Variability in soybean agronomic performance traits in response to 41°C heat and high relative humidity seed stress

by

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ABSTRACT

Inbreeding plant species have shown genetic variability; and the sources for this variability can be the result of plant stress, growing environment, residual heterozygosity, or *de novo* variation. The accelerated aging seed vigor test is a seed stress test but, to our knowledge, has not been used immediately prior to planting as a form of seed stress, and to exploit intra-cultivar variability in established soybean lines. The objectives of this study were to evaluate if a modified version of the accelerated aging test as a seed-stress technique would exploit intra-cultivar variation in soybean agronomic traits of plant stand, plant height, plant maturity, yield, seed size, seed protein and oil content, and seed standard germination and vigor; and to determine if the agronomic performance changes were stable, or heritable, over multiple years and locations. Seed of two soybean cultivars, 'BSR 101' and 'Jack' were stressed using a modified version of the accelerated aging protocol, at 41°C for 48 hours, after which seeds immediately were hand-planted in the field. The agronomic traits of non-stressed and stressed seeds were measured in the first and second generation plots. The first generation plots were planted near Ames, Iowa for two growing seasons, and the second generation plots were planted in same growing seasons at two locations, near Ames, Iowa, and Missouri Valley, Iowa. In 2010, cultivars exhibited greater variance and higher means in seed yield in response to the seed-stress treatment. When second generation plots were evaluated, variance did not change for all traits, but both cultivars had an increase in mean plant stand. In 2011, variance did not change for all traits in response to seed stress, but mean values decreased in all traits. In the second generation plots, there was a mean increase in plant stand and seed yield, but variance did not change. Results were subject to genotype by

environment interactions, causing differences between growing seasons and cultivars. Intra-cultivar variation has been utilized to select for superior germplasm. Changes in variance and increased performance of agronomic traits can be used to select superior lines from within already established soybean cultivars.

CHAPTER 1: GENERAL INTRODUCTION

Literature Review

Organisms which inbreed, or self pollinate, often have low levels of genetic variability, which can limit the breeding of improved cultivars. Genetic variation has been reported in inbreeding plant species (Rasmusson and Phillips, 1997; Buckler and Thornsberry, 2002; Lolle et al., 2005), and this variation within species could be used in breeding (Phillips, 2010). Various reasons have been cited for generation of this genetic variation; plant stress, growing environment, or *de novo* variation (Schneeberger and Cullis, 1991; Madlung and Comai, 2004; Phillips, 2010;). This review will discuss the existence of genetic variation within plant species, the generation of variation in plants in response to growing environment or plant stress, and the effect of plant and seed stress on phenotypic variation and agronomic performance traits of plants.

Genetic Variation

Existence of genetic variation has become easier to document due to advances in molecular biology. Scientists now have the ability to sequence large genomes, which has led to the hypothesis that genomes are not fixed, and that plant genomes in particular seem to be relatively fluid. This allows for large differences in size and organization to occur even in closely related species (Casacuberta and Puigdomenech, 2000). Major differences occurred in random genes within a single genome (Buckler and Thornsberry, 2002). Genetic variation, which was not present in the parent lines, existed in the progeny, and was called *de novo*

variation (Phillips, 2010). Mechanisms through which synthesis of *de novo* variation occurred include; point mutations, intragenic recombination, transposable elements, epigenetic variation, gene amplification, and others (Rasmusson and Phillips, 1997).

As an inbreeding plant, soybean [*Glycine max* (L.) Merr] is generally homozygous. Roth et al., (1989) found that soybean exhibited genetic variation in restriction fragment length polymorphisms (RFLPs). In general, RFLPs showed that soybean species contained two alleles at any single locus. When cultures were prepared from root and leaf tissue, RFLP analyses showed that there were differences at various loci in the root tissue, generally in the form of an additional fragment, suggesting that genetic variation occurred from recombination events.

High rates of intra-cultivar structural variation were observed in soybean cultivar 'Williams 82' (Haun et al., 2011), which was derived from a cross between 'Kingwa' and 'Williams'. Variation was exhibited in the number and size of introgressed 'Kingwa' loci. The high rates of intra-cultivar variation were the result of structural variation between the parental lines (Haun et al., 2011).

In Arabidopsis (*Arabidopsis thaliana*), progeny plants contained DNA that was not present in the parents (Lolle et al., 2005). Plants homozygous recessive for the *HOTHEAD* (*hth*) allele were crossed with wild-type plants homozygous for (*HTH*). The expected progeny would be heterozygous (*HTH/hth*), but 8 of 164 embryos were homozygous (*HTH/HTH*). This suggested that (*hth/hth*) plants were a source of pollen for the (*HTH*) allele, which resulted in a progeny genotype that was not present in the genome of the parents. Similarly, in tobacco (*Nicotiana tabacum*), DNA increased in doubled haploid lines

in the progeny, while chromosome number remained the same (Reed and Wernsman, 1989). These studies cite *de novo* variation as the cause of variation in the progeny plants.

In maize inbred lines, genomic changes were reported as a result of mutations, which led to progeny lines different than the parents (Guo et al., 2004). Genomic variation was expressed in allelic expression levels. The expected level of allelic expression would be an equal 1:1 = 1.0, however, in 9 of 15 genes analyzed, there was a significant ($P \leq 0.05$) deviation from 1.0 (Guo et al., 2004). Russell et al., (1963) also reported genome changes, thought to be the result of mutations, which led to progeny lines different than the parents. Buckler and Thornsberry, (2002) found genetic variation in maize genomes. They attributed the variation to polymorphisms, in which 21 loci varied by as much as 16-fold.

Stress Induced Plant Variation

Stress is a cause of genetic variation in plants (McClintock, 1984; Ries et al., 2000; Guo et al., 2004; Molinier et al., 2006). Stress is defined as any form of disequilibrium for the plant (Walbot and Cullis, 1985), which impacts it not only through physiological responses, but through generation of genomic and epigenetic responses (Madlung and Comai, 2004).

Plant Growing Environment

LACK OF MOBILITY. Plants are immobile; consequently, they must develop mechanisms or processes by which they adapt to changes in their surroundings. Immediate

responses to environmental stresses occur in the form of regulation of physiological processes, but, over time, plants may adapt or create variation in order to develop a long-term stress response (Walbot and Cullis, 1985). Plant genomes remain fluid to adapt to environmental changes. The plant's ability to adapt to the growing environment is a potential reason why they have high levels of genetic diversity, which enables them to survive when they cannot move from their location of germination (Hamrick et al., 1987).

GROWING ENVIRONMENT. Plants responded to certain stresses or challenges of the growing environment, even if not prepared for them. This response was attributed to a rearrangement or reorganization of the genome, which enabled the plant to survive a stress condition (McClintock, 1984). Heritable genomic changes have been reported in flax (*Linum usitatissimum*), in response to environmental cues. Differences were observed in plant weight and attributed to permanent changes in the amount of nuclear DNA in response to changes in the growing environment (Evans et al., 1966).

Schneeberger and Cullis (1991) also reported genomic changes in flax in response to growing environments. A 5S rRNA gene probe was used to identify RFLPs. When plants grown in different environments were compared, there was a high degree of heterogeneity at the 5S rRNA gene sequence, and the results were highly variable between locations (Schneeberger and Cullis, 1991). Chen et al. (2005) later reported that these changes in RFLPs were stable, or heritable, after the initial change.

The meristematic cells of flax plants showed DNA changes during vegetative growth, prior to flowering, attributed to growing environment (Cullis, 2005). These changes were either stable or unstable, and the specific growing environment defined the phenotypic and

genotypic expression of the first generation progeny. Stable inheritance in subsequent generations was more likely if plants were self-fertilized (Cullis, 2005).

Arabidopsis plants grown under a high salt environment of 0.1 M NaCl , had more than a 2-fold increase in recombination frequency compared to the control, indicating that the Arabidopsis genome adapted to the growing environment (Puchta et al., 1995). DeBolt (2010) reported that Arabidopsis plants displayed *de novo* genomic structural variation when grown under stressful conditions. Plants were stressed for five generations using salicylic acid spray. Genomic hybridization was used to identify regions of gene-copy number variation between the stressed and non-stressed plants. Those grown under the stress conditions exhibited increased amounts of gene-copy number variation after five generations (DeBolt, 2010).

Maize plants grown in different environments had genetic differences, in which 5 of 15 genes analyzed exhibited different amounts of allelic expression as analyzed by RT-PCR, and stress responsive genes had the greatest amount of variation (Guo et al., 2004).

Honeycomb is a planting design in which single seeds are spaced equidistant from each other, and wide-spaced, to maximize phenotypic expression by maximizing genotype by environment (G X E) interactions, and minimizing plant-to-plant competition. The isolation environment matches the ideal conditions for plant growth because it nullifies interferences among plants and provides equal sharing of resources (Fasoula and Faoula, 1997). The honeycomb design enables the use of many plant replicates and is designed to test the G X E interactions by sampling effectively for environmental heterogeneity (Fasoula and Fasoula, 1997). Soybean cultivars exhibited large amounts of variation in response to the honeycomb

planting design, which allowed for selection of multiple new germplasm lines from three cultivars, ‘Benning’, ‘Cook’, and ‘Haskell’ (Fasoula et al., 2007a, b, c).

Variation within soybean cultivars (Fasoula et al., 2007 a,b,c) was attributed to residual heterozygosity (Yates et al., 2012). The new germplasm lines released by Fasoula et al. (2007a, b, c) were phenotypically and genotypically unique, as analyzed by SSR makers. In soybean cultivars ‘Benning’, ‘Haskell’, and ‘Cook’, 82, 93, and 82% of the variation detected in single-plant lines, respectively, was in the original foundation seed source (Yates et al., 2012).

When planted in a wide-space planting, or honeycomb design, three varieties of tomato (*Lycopersicon esculentum*) expressed variation freely, enabling selection of superior plants. In two of three varieties, selection of the superior phenotypes translated into a substantial yield increase as early as the F_3 generation, and the selected plants performed better than the F_1 hybrids (Christakis and Fasoulas, 2002).

In climbing dry bean (*Phaseolus vulgaris* L.), the honeycomb design enhanced yield (Tokatlidis et al., 2010). The absence of competition enabled maximum phenotypic expression and selection of exceptional genotypes, with an average yield improvement of 5% and 4% in two traditional dry bean landrace populations. The within-cultivar genetic variation was used for the advancement of superior lines (Tokatlidis et al., 2010).

Sunflower (*Helianthus annuus*) seedlings exposed to high temperature stress had greater variability than seedlings not exposed to stress. Under high temperature stress, seedlings exhibited recovery growth, particularly in the measured traits of root and shoot length. Recovery growth was highly variable within the population. In non-stressed

seedlings, the variability in root and shoot length was not present (Senthil-Kumar et al., 2003).

Genetic differences in wheat (*Triticum aestivum*) occurred in response to temperature stress. Cultivars were administered either no treatment, or a heat-shock treatment. After the initial heat-shock treatment, both the untreated and treated seedlings were exposed to a lethal temperature. In the untreated group, complete loss of cell viability occurred within 60 minutes, while in the treated group plants maintained 50 to 80% cell viability. This difference was attributed to genetic differences in cell viability induced by the heat-shock treatment (Krishnan et al., 1989).

Genome stability was measured in tobacco and Arabidopsis plants exposed to elevated UV-B radiation by assaying homologous recombination events in treated plants and their progeny. All lines changed morphologically in response to UV-B exposure, and a higher frequency of genetic recombination events was observed in response to increased levels of UV-B radiation exposure (Ries et al., 2000). The frequency of genetic recombination events was different in all lines, suggesting that recombination events in response to UV-B radiation were genome dependent. Exposed to the same level of UV-B radiation as the parents, F1 and F2 progeny plants had a 2.4-fold and 3.2-fold increase in genetic recombination frequency, respectively, than the parents. This suggested that the number of plants with permanent genetic recombination events increased with the level of the UV-B radiation and with each progeny generation (Ries et al., 2000).

Kovalchuck et al. (2000) reported a variable frequency of point mutations in the Arabidopsis genome in response to stress. Transgenic plants, which prevented the translation of the active *uidA* gene, were used to test the reversion frequency back to the active *uidA*

gene. Spontaneous restoration of the active *uidA* gene was observed in higher frequencies than estimated, exceeding estimations by as much as 100-fold. Additionally, a large variation in mutation frequency occurred in response to different levels of various DNA damaging agents, including UV-C radiation, X-rays, and methyl methanesulfonate. All DNA damaging agents increased the amount of reversion frequency when compared to plants without an additional stress (Kovalchuk et al., 2000).

Similarly, application of chemicals that prevented DNA repair in Arabidopsis increased the frequency of homologous recombination (Puchta et al., 1995). UV light and methyl methanesulfonate (MMS) were applied to prevent DNA repair, which disrupted a β-glucuronidase marker gene. The disrupted β-glucuronidase was used to analyze the frequency of the intrachromosomal homologous recombination, which was enhanced several-fold by these DNA damaging agents (Puchta et al., 1995).

Arabidopsis plants, carrying a *β-glucuronidase* marker gene as recombination substrate, were used to monitor genetic variation in response to environmental stress (Kovalchuck et al., 1998). The homologous recombination frequency was studied in plants grown on radioactive soil from the Chernobyl atomic disaster site. A significant increase in somatic intra-chromosomal recombination frequency was observed, suggesting that plants had genetic mechanisms to adapt to non-typical environmental stress (Kovalchuck et al., 1998).

Stress-induced genome-wide changes in Arabidopsis plants persisted through several generations (Molinier et al., 2006). Six lines were treated with two stresses of the short-wave radiation UV-C, or flagellin. Both stresses increased the frequency of somatic homologous recombination in all lines studied. Additionally, the progeny of all lines showed a 2- to 4-fold

increase in the homologous recombination frequency in response to stress, when compared to progeny of the non-treated plants. The increases in homologous recombination frequency in the progeny suggested that plants had heritable genome flexibility, increasing the potential for adaptation (Molinier et al., 2006).

BIOTIC STRESS. Pathogens have a similar stress impact on plants as non-living stressors (Mitrick et al., 1985; Lucht et al., 2002; Kovalchuck et al., 2003). Homologous recombination frequency was monitored in virus infected tobacco plants. Plants were exposed to the virus through inoculation of plant leaves, in which leaves on the same plant were both inoculated with the virus, or not inculcated. The virus entered the non-inoculated plant leaves approximately 24 to 36 hours after the initial infection, but the frequency of recombination events in the non-inoculated plant parts increased only eight hours after the initial infection. This rapid response suggested that the signal for induction of homologous recombination traveled to new leaves faster than the virus itself (Kovalchuk et al., 2003).

In Arabidopsis, an increase in recombination frequency was reported due to infection with a pathogen (Lucht et al., 2000). Plants infected with the pathogen *Peronospora parasitica* expressed a 1.8-fold increase in the recombination frequency when compared to that of uninfected plants. Results indicated that Arabidopsis plants adapted to stress through genetic variation (Lucht et al., 2002).

Maize plants infected with barley stripe mosaic virus, BSMV, had genomic changes through insertion of the transposable element *Bs1*. The insertion of the mutant S5446, which caused the *Bs1* sequence, was recovered in the progeny of plants infected with BSMV, indicating that a heritable genomic change occurred (Mitrick et al., 1985).

Retrotransposons in tobacco exhibited a selective response to pathogen infection (Grandbastien et al., 2005). The *Tnt1* retrotransposon of the Solanaceae family, which is activated by microbial factors, was discovered in tobacco. There were large amounts of variability in expression of *Tn1* in response to different stress conditions (Grandbastien et al., 2005).

Genetic Variation or Stress Conditions Impacting Plant Phenotype and Agronomic Performance

Plant Phenotype

Although the mechanisms for genomic variation are not understood, it is clear that expression of variation in plant genotypes can have profound effects on the phenotype (Phillips, 2010). Stressful environments altered the way in which plants expressed morphological features, and ultimately affected agronomic performance (Madlung and Comai, 2004). Differences in genetic variation in the form of allelic expression different than the expected 1.0:1.0 ratio in maize contributed to phenotypic variation (Guo, et al., 2004). Variability of the environments in which soybeans were grown contributed to large amounts of phenotypic variation in parental single plants grown in a row (Green and Pinnell, 1968). Phenotypic intra-cultivar variation was observed in soybeans planted in a honeycomb design (Fasoula and Boerma, 2007).

Agronomic Performance

Genetic and phenotypic variation can cause changes in the agronomic performance traits of plants (Russell et al., 1963; Fehr and Probst 1971; Rasmusson and Phillips, 1997; Munamava et al., 2004; Cullis, 2005). In flax, changes in the DNA caused variation in maturity, plant height, and the peroxidase enzyme (Cullis, 2005). In maize, seed germination and vigor were different depending on inbred adaptation to the growing environment. Seed germination of maize inbreds produced in cooler climates had higher average cold temperature germination and higher seedling vigor. Inbreds achieved maximum seed germination and vigor when grown in environments with similar climatic conditions to those where the seed was produced or breed (Munamava et al., 2004). When maize was classified based on agronomic performance parameters into groups according to mutants, progeny lines were often in different groups than their parents (Russell et al., 1963). In barley, cultivars with the greatest amount of genetic variability showed the greatest amount of gain in agronomic performance (Rasmusson and Phillips, 1997).

Arabidopsis plants contained variation in reproductive traits (Shaw, 2010). Number of seeds per fruit and number of fruits, were measured in 120 lines of Arabidopsis advanced for 17 generations. The means did not different among generations, but by the 17^{th} generation, there was significant variation among lines (Shaw, 2010).

Growing environment caused significant changes in agronomic and chemical performance traits in soybean. A wide-spaced, or honeycomb planting design, allowed selection of five, seven, and six new germplasm lines from cultivars ‘Benning’, ‘Haskell’, and ‘Cook’, through expression of variation in the agronomic traits of seed protein and oil

content, seed weight, plant height, and maturity (Fasoula et al., 2007a, b, c). Within three cultivars, single-plant progeny lines were selected from the wide-spaced honeycomb design due to variation in seed weight, maturity, plant height, and lodging (Fasoula and Boerma, 2007). Differences were found in soybean lines by growing environment interaction in traits of maturity and field emergence percentage. Location by seed source interaction showed significant differences in yield, field emergence percentage, and seed protein content (Fehr and Probst, 1971).

High temperature and drought stress impacted seed chemical composition in soybean (Dornbos and Mullen, 1992). As the number of days under drought stress increased, seed protein increased linearly and oil decreased linearly. In a severe drought stress situation, protein content increased by up to 4.4 percent and oil content decreased by 2.9 percent. Temperature stress also impacted the protein and oil content of the seeds. When seeds were produced under a daytime temperature stress of 35°C, seeds contained 4 percent more protein and 2.6 percent less oil than when produced at 29°C (Dornbos and Mullen, 1992). Thomas et al. (2003) found significant differences in soybean seed composition due to plant exposure to different growth temperatures. Oil content in the seed was highest when plants were grown at a daytime/nighttime temperature cycle of 32 / 22°C for the entire plant-life cycle, and decreased at higher temperatures. Oleic acid increased with higher temperature, while linolenic acid decreased. Nitrogen and phosphorus increased in the seed up to daytime/nighttime temperature cycles of 40 / 30°C, but decreased at higher temperatures (Thomas et al., 2003).

Seed protein and oil content differences were reported in soybeans at four different locations in Argentina (Maestri et al., 1998). Seed oil content ranged from 198 to 267 g kg^{-1}

of dry matter, and seed protein content ranged from 377 to 436 g kg^{-1} of dry matter. Mean values of protein and oil contents were significantly different for the same cultivar grown at different locations. Significant variations were observed among environments for the majority of soybean chemical parameters evaluated (Maestri et al., 1998).

The seed oil and protein content of soybeans was significantly affected by genotype, location, and genotype by location at four different locations in India. Protein content ranged from 32.2 to 42.1%, while oil content ranged from 15.4 to 22.0%. All genotypes showed significant variation for unsaturated fatty acid profiles over the four locations. Seed size also was different among genotypes and genotype × locations (Kumar et al., 2006).

Seed Stress Impacts on Genome and Agronomic Performance

Accelerated Aging Seed Stress

The accelerated aging test is used to test soybean seed vigor [the Association of Official Seed Analysts (AOSA, 2002)]. This test uses two sources of seed stress, high temperature of 41°C and high relative humidity. During imbibition, accelerating aging decreased the early respiration of hydrated cotyledons, decreased dry matter of the cotyledons, and increased electrolyte leakage into the aging medium (Parrish and Leopold, 1978). Seed germination, root length and axis weight also decreased as time of accelerated aging increased, while oxygen consumption decreased and electrolyte leakage increased. The decrease in oxygen consumption and increase electrolyte leakage were evidence that

membrane integrity of the seed cells was compromised during the aging process (Parrish and Leopold, 1978).

Accelerated aging negatively impacted the ability of seeds to germinate (Robert et al., 1979; Hsu et al., 2003). During aging, the saturation of polar lipids and imbibitional damage during rehydration increased. Peroxidation of unsaturated fatty acids occurred during aging at high relative humidity as the seeds deteriorated. When seeds were aged at high humidity, they were unable to synthesize superoxide dismutase after imbibition, and free radical chain reactions were uncontrolled, causing the aging damage to be compounded (Robert et al., 1979). Similarly, in bitter gourd (*Momordica charantia* L.) seeds, accelerated aging increased lipid peroxidation and decreased activity of free radicals scavengers. Aged seeds had lower emergence when compared to non-aged seeds (Hsu et al., 2003).

Seed Stress, Genetic Variability and Agronomic Performance

Seed stress impacted morphological traits and genetic expression in crops (Matlock 1953; Green et al., 1966; Keigley and Mullen, 1986; Ashan et al., 2007). Using a two-dimensional electrophoresis technique, rice seeds were germinated under toxic copper concentration and seedlings were evaluated for protein concentration by analyzing the number of protein stains. Using this technique, 25-protein stains were found. Eighteen of these protein stains were upregulated in response to high copper seed stress, while 7 were downregulated, suggesting that plants exhibited a genetic response to seed stress (Ahsan et al., 2007). Additionally, rice seedlings exhibited changes in protein content due to exposure

to excess copper during seed germination and germination rate, shoot elongation, plant biomass, and water content decreased (Ahsan et al., 2007).

Seed mechanical damage negatively affected seed germination in the laboratory and the field. Combine-harvesting at high seed moisture content increased seed-coat damage, and decreased the percentage of field emergence. Hand-shelling the high moisture seed resulted in less seed damage, and produced higher seed germination percentages and fewer abnormal seeds. Larger seeds were more susceptible to mechanical damage than smaller seeds (Green et al., 1966). When seeds were divided into categories of none, slight, and severe seed coat damage, mean germination percentages in the laboratory were 80%, 58%, and 45%, respectively. Field emergence of these seed-coat damage categories was 52%, 20%, and 12%, respectively (Matlock, 1953).

Soybean seed quality deteriorated due to high temperature stress during seed-filling (Keigley and Mullen, 1986). Laboratory germination and vigor percentage and physical seed quality declined linearly in response to high temperatures during the seed-filling period, even when high temperature exposure was limited to the first 10 to 13 days of seed fill (Keigley and Mullen, 1986).

Importance of Genetic Variation

Traditional breeding programs, where an elite inbred line is crossed with another elite inbred line, were thought to narrow the genetic base, and the amount of variability that existed within cultivars (Rasmussen and Phillips, 1997). Efforts to improve plant tolerance to stresses have been limited because of the complexity of the genes and because traditional

breeding methods limit genetic variability (Cushman and Bohert, 2000). However, genetic variation has been found in inbreeding plant species (Rasmusson and Phillips, 1997; Buckler and Thornsberry, 2002; Lolle, et al., 2005), and could have important implications for plant breeding (Phillips, 2010). Breeding programs depend on germplasm diversity for success (Johal, et al., 2008) and this diversity could have a large role in increasing yield (Phillips, 2010). To improve crops, it is essential to use this variation, and sort through the diversity to find the alleles and polymorphisms that are beneficial (Buckler and Thronsberry, 2002).

Variability in soybeans was used to improve agronomic performance traits such as yield, seed protein and oil content, and plant height, which enabled the selection of new germplasm (Fasoula et al., 2007 a, b, c). If utilized, variability can not only help improve yield and other agronomic performance traits, but also improve plant tolerance to stress and to global changes in the environment. Exploiting existing variation can help identify new genes and their roles in responses to stress (Cushman and Bohnert, 2000). Research into how plants implement survival mechanisms in stress conditions provides a better understanding of the diversity of plant species, and how this diversity can be used to positively impact agricultural productivity and economics (Amtmann et al., 2005).

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Thesis Organization

Chapter two is written in manuscript format for submission to a scientific journal. This paper is titled “Variability in agronomic performance traits of soybean in response to 41°C heat and high relative humidity seed stress”. Chapter 3 is a general summary and discussion. References, tables, and figures have been included at the end of each chapter.

CHAPTER 2: VARIABILITY IN SOYBEAN AGRONOMIC PERFORMANCE TRAITS IN RESPONSE TO 41°C HEAT AND HIGH RELATIVE HUMIDITY SEED STRESS

A paper to be submitted to *Crop Science*

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Abstract

Incorporation of genetic diversity into soybean [*Glycine max* (L.) Merr.] cultivars can be used to increase yield and plant adaptation to stresses. Genetic variation has been found in commercial soybean cultivars, but using a seed stress to generate intra-cultivar variation has not been studied. The first objective of this study was to use seed stress in an attempt to generate variation in soybean agronomic traits of field stand, plant maturity, plant height, seed size, seed yield, seed protein content, seed oil content, seed standard germination and seed vigor. The second objective was to evaluate if changes in variation were heritable. Seed of two soybean cultivars, 'BSR 101' and 'Jack' were stressed using a modified version of the accelerated aging protocol, at 41°C for 48 hours, after which seeds immediately were hand-planted in the field near Ames, Iowa. Heritability of agronomic traits in the second generation was measured in two growing seasons, and at two locations, near Ames, Iowa, and Missouri Valley, Iowa. In 2010, cultivars exhibited increased variance and a mean increase in seed yield in response to the seed-stress treatment. In second generation plots

variance did not change for all traits, but both cultivars had a mean increase in plant stand. In 2011, variance did not change for all traits in response to seed stress, but mean values decreased in all traits. In the second generation plots, there was a mean increase in plant stand and seed yield, but variance did not change. Results were subject to genotype by environment interactions, causing differences between growing seasons and cultivars. We found increase variation, large ranges in the agronomic trait values within treatments, and increased performance of agronomic traits in response to a seed stress treatment. This treatment can therefore be used to induce variation, which will aid in selection of superior plants from within already established soybean cultivars, to improve yield and other agronomic traits.

Introduction

Self-pollinated or autogamous plant species, such as soybean [*Glycine max* (L.) Merr.] often lack genetic variation. Most traditional breeding programs have limited the amount of genetic variability in cultivars, by narrowing the genetic base (Rasmusson and Phillips, 1997; Cushman and Bohnert, 2000). However, evidence of genetic variation has been reported in autogamous plant species (Schneeberger and Cullis, 1991; Lolle et al., 2005; Phillips, 2010) because plant genomes are not stable, but fluid (Casacuberta and Puigdomenech, 2000). Incorporation of genetically diverse germplasm into established cultivars could improve plant adaptation to stress and improve yield (Johal et al., 2008; Phillips, 2010). Genetic diversity could also lead to the identification of novel genes (Cushman and Bohnert, 2000).

Roth et al. (1989) found genetic variation in restriction fragment length polymorphisms (RFLPs), generally in the form of an additional fragment, in soybean tissue

cultures generated from root tissue, when compared to those generated from leaf tissue. In arabidopsis (*Arabidopsis thaliana*), the progeny from a cross between plants homozygous for the *HOTHEAD* (*hth*) alleles and the wildtype (*HTH*) alleles, produced homozygous individuals for the wildtype alleles (*HTH/HTH*), showing a reversion to the alleles present in only one of the parent plants (Lolle et al., 2005). Genetic changes have been found in the progeny of crosses of doubled haploid lines of tobacco [*Nicotiana tabacum*(L.)], in which chromosome number remained the same but the amount of DNA increased, as a result of *de novo* variation (Reed and Wernsman, 1989). In flax species, heritable changes in response to environmental cues were through divergence from the majority of the 5S rRNA gene (Schneeberger and Cullis, 1991; Cullis, 2005).

Variation in the agronomic traits of plant height, plant maturity, seed protein and oil content, and yield have been reported in field plants grown in a wide-spaced, or honeycomb design (Fasoula and Fasoula, 1997; Christakis and Fasoula, 2002; Fasoula and Boerma, 2007; Fasoula et al., 2007). The wide-spaced growing environment contributed to genetic differences by maximizing phenotypic expression and exploiting the genotype by environmental interaction (Fasoula and Fasoula, 1997). In hybrid tomato plants, wide-spaced planting increased the amount of variation in yield, which was used to select for superior plants from lines that were supposedly homozygous (Christakis and Fasoulas, 2002). Three commercial cultivars of soybean planted in a honeycomb or wide-spaced planting design exhibited intra-cultivar variation in seed protein and oil content, and fatty acid composition (Fasoula and Boerma, 2007). The variation in agronomic traits was heritable and resulted in the release of multiple new germplasm lines from cultivars ‘Benning’, ‘Haskell’, and ‘Cook’ (Fasoula et al., 2007a; 2007b; 2007c).

Multiple theories have been postulated about the generation of intra-cultivar variation. Research conducted by Lolle et al. (2005) led to the theory that genetic variation was the result of template RNA stored in plants from previous generations, which was maintained for several successive generations. Research suggested that metabolic stress generated in mutants increased the reversion frequency in Arabidopsis, creating genetic diversity. In barley (*Hordeum vulgare* L.) genetic variation was thought to be the result of point mutations, intragenic recombination, transposable elements, epigenetic variation, gene amplification, or structural changes (Rasmusson and Phillips, 1997).

Yates et al. (2012) reported that residual heterozygosity contributed to genetic variation within soybean germplasm lines released from cultivars 'Benning', 'Haskell', and 'Cook'. Similarly, Haun et al. (2011) reported that structural genomic variation among different individual plants from within soybean cultivar 'Williams 82' was the result of residual heterozygosity. *De novo* generated alleles were credited as a significant source of variation in inbred lines (Rasmusson and Phillips, 1997; Phillips, 2010). *De novo* variation is genetic variation present in the progeny that was not present in the parental generations (Rasmusson and Phillips, 1997).

The first objective of this study was to use seed stress in an attempt to increase the amount of phenotypic variation in two inbred soybean cultivars. This variation could then be used to select for superior lines. The second object was to determine if variation created in inbred lines was heritable, or stable, over multiple years and generations. Heritable variation may imply that genetic variation exists within inbred soybean lines, and is expressed when conditions are correct. Favorable, heritable variation could be used to produce new cultivars which could increase yield potential and tolerance to certain environmental conditions.

Materials and Methods

Seed source and handling

PARENT MATERIAL. Soybean cultivars 'BSR 101' (PI 548519) and 'Jack' (PI 540556) were used in this study. 'BSR101' was developed for its resistance to the disease brown stem rot, caused by *Phialophora gregata* (Allington and Chamberlain) W. Gams (Tachibana et al., 1987). 'Jack' was developed by the Illinois Agricultural Experiment Station in 1989 for its resistance to soybean cyst nematode (*Heterodera glycines* Inchinohe) (Races 3 and 4) (Nickell et al., 1990).

SEED MATERIAL. In the first growing season, 'BSR 101' and 'Jack' parent seeds were planted in a wide-spaced, or honeycomb, planting design (Fasoula and Fasoula, 1997), with four seeds planted per 2 m space to eliminate negative impacts of competition. After emergence, seedlings were thinned to one plant per space. Each individual plant was given a different code, termed 'entry', which was maintained throughout the duration of the study. Plants, or entries, were harvested separately and single-plant threshed, to minimize contamination by other entries. The next season, seed from each individual entry were tractor-planted in rows to increase seed. The following season, seeds from individual entries of the seed increase were used in the seed-stress experiment (Fig. 1). Progeny seeds harvested from individual entries were planted the following season in a heritability, or second generation plot (Fig. 1), in which no seeds were treated.

EXPERIMENTAL DESIGN. The seed-stress experiments were planted during the 2009, 2010, and 2011 growing seasons. Seeds were planted in the field in a split-plot design. The main effect was field repetition, followed by cultivar, and by treatment. Seeds were planted

at a rate of 50 seeds per entry in a 2.2 m row. Sixty-four entries of each cultivar were planted in 2009, 20 entries of each cultivar in 2010, and 64 entries of each cultivar in 2011.

In 2010 and 2011, second generation, or progeny, seeds from the 2009 and 2010 growing seasons were planted to test for heritable changes in agronomic traits. Plots were planted in a split-plot design in two locations, near Ames, Iowa and Missouri Valley, Iowa. The main effect was location, followed by field repetition, cultivar, and treatment.

SEED STRESS TREATMENT. Seeds from each entry were given a stress treatment, or not stressed and used as a control. The stress treatment was administered by using a modified version of the Association of Official Seed Analyst accelerated aging protocol (AOSA, 2002). Fifty seeds of each entry were placed in a single layer on top of a wire-mesh screen suspended over 40 mL of distilled water inside a clear plastic box (11 × 11 × 3.5 cm) (Hoffman Manufacturing Company, Albany, OR). Boxes were placed in a water jacketed accelerated aging chamber at 41 °C, for 48 hours. After removal from the chamber, the moist seeds were transported to the field. Stressed and non-stressed seeds were carefully hand-planted, equally spaced in a furrow, and covered gently with soil within two hours.

Agronomic traits data

Agronomic traits evaluated were field stand, plant maturity, plant height, seed size, seed yield, seed protein and oil content, and standard germination, and accelerated aging of the progeny seeds (AOSA, 2002; 2009).

FIELD STAND. The number of plants per entry that emerged were counted and recorded approximately three weeks after emergence.

PLANT MATURITY. Plant maturity was defined as the date when all plants in the entry were no longer green. Single-entry ratings were transformed into the number of days to plant

maturity after 1 September. This date was arbitrarily picked as the zero rating because no entries matured earlier than 1 September.

PLANT HEIGHT. Plant height was the average of three randomly selected plants from each entry, measured in centimeters, as the distance from the soil to the top of the main branch of the plant.

SEED SIZE. Seed size was reported in grams, as the average of two 100-seed samples.

SEED YIELD. Seed yield for each entry was recorded in the non-stressed and stressed entries after the seeds were cleaned, and reported as the total weight of seeds harvested in grams. In the second generation plots, yield was obtained from the combine monitor, calculated from the weight and moisture of each entry, and reported in kg ha^{-1}.

SEED PROTEIN AND OIL. Seed protein and oil analysis were performed by using near-infrared transmittance. In 2010, tests were conducted by the USDA-ARS, National Center for Agriculture Utilization and Research in Peoria, Illinois. In 2011, tests were conducted at the Iowa State University Grain Quality Laboratory in Ames, Iowa.

STANDARD GERMINATION. Standard germination tests were conducted according to AOSA rules for testing seeds (AOSA, 2009). Four entries, of 100 seeds each, were planted on moistened crepe cellulose paper (Kimberly Clark, Neenah, WI). Samples were germinated in a controlled growth environment at 20 °C for seven days, and then classified as normal seedlings, abnormal seedlings, or dead seeds according to the AOSA rules (2009).

ACCELERATED AGING. Accelerated aging tests were conducted according to the AOSA protocol (AOSA, 2002) for testing soybean seed vigor. One hundred seeds per entry were placed in a single layer on a wire-mesh screen suspended above 40 mL of water inside an accelerated aging box (11 × 11 × 3.5 cm) (Hoffman Manufacturing Company, Albany,

OR). Boxes were placed in a water jacketed accelerated aging chamber at 41 °C for 72 hours. Upon removal from the chamber, seeds were planted on moistened crepe cellulose paper (Kimberly Clark, Neenah, WI) and covered with 2.5 cm of moist sand. Seeds were allowed to germinate in a controlled environment at 20 °C for seven days, after which they were evaluated according to the AOSA protocol (2009).

Data analysis

Variance within each treatment was calculated as the sum of each entry minus the mean, squared and divided by the number of entries in each sample. The formula for variance is as follows: $\frac{\sum (Yi - \bar{Y})^2}{n - 1}$.

Changes in variance between treatments were compared using Hartley's F-max test. Treatments were compared using a generalized linear model (GLM) procedure (SAS Institute, Inc., 2009), and were analyzed as a split-plot design. Years and cultivars were analyzed separately to evaluate all changes in variance.

Results

Seed Moisture

Before seed stress, both cultivars were at a seed moisture content of approximately 0.07 g water g^{-1} seed. After exposing seeds to the accelerated aging test for 48 hours, 'BSR 101' seed moisture content was 0.31 g water g^{-1} seed, and 'Jack' seed moisture content was 0.33 g water g^{-1} seed (Table 1).

Non-stressed and stressed plots

In the 2010 growing season, plants grown from stressed seeds of 'BSR 101' were 11.06 cm shorter, they yielded 9% higher, and their yield variance increased by 94%. The difference between the highest and lowest yielding entries was 311 g. In progeny seeds of

plants grown from stressed seeds, oil content was 0.6% lower, and oil content variance increased by 73%. The range between the highest and lowest seed oil contents was 2.47%. Average seed size was the same between treatments, but seed size variance increased by 67%. In plants grown from the stressed seeds, progeny seed standard germination and vigor tests improved by 14% and 36%, respectively (Table 2).

Plants grown from the stressed seeds of 'Jack' were 10 cm shorter, and height variance decreased by 69%. Yield increased 23%, and yield variance increased 86%. The difference between the highest and lowest yielding plants grown from stressed seeds was 427 g. In the progeny seeds of plants grown from stressed seeds, seed protein content increased 1% and seed oil content decreased 3%. Standard germination and vigor improved by 14% and 17%, respectively, in the progeny seed of plants grown from the stressed seeds (Table 2).

In the 2011 growing season, variance between the stressed and non-stressed seeds was not different for all traits measured, in both cultivars, but mean differences occurred. In plants grown from the stressed seeds of 'BSR 101', plant stand decreased by 19%, and plant height decreased by 2 cm. Plants grown from stressed seeds maturated approximately 1 day later than those grown from non-stressed seed, and the range in days to maturity was 12 days. Yield of 'BSR 101' plants grown from stressed seeds decreased by 10%. The difference between the highest and lowest yielding entries was 390 g. Progeny seed size decreased by 1% in the plants grown from the stressed seeds (Table 2).

In plants grown from the stressed seeds of 'Jack', plant stand decreased by 2%, and plant height decreased by 3 cm. Seed yield decreased by 8% in the plants grown from the stressed seed, and the range in yield values was 960 to 280 g (Table 2).

Second generation plots

Second generation plots were those grown from the progeny seeds of the non-stressed and stressed seeds of the previous season. There were no differences in variance in the 2010 growing season, but mean differences occurred. In the progeny of the stressed seeds in 'BSR 101', plant stand improved by an average of 31 plants, and the range in plant stand was 90 plants. Progeny plants of the stressed seeds matured 1.5 days earlier than those grown from the non-stressed seeds in 'BSR 101', and seed size was 2% larger. The range in days to maturity was 18 days in the plants grown from the progeny of the stressed seeds, and 12 days in the progeny grown from the non-stressed seeds. The difference in yield between the highest and lowest yielding entries in plants grown from the progeny of stressed seeds was 3004 kg ha^{-1} (Table 3).

In the progeny of the stressed seeds in 'Jack', plant stand improved by 27 plants, and the range in plant stand was 150 plants. Progeny plants of the stressed seeds matured approximately 1 day later, and the range in days to maturity was 9 days. Yield of plants grown from the progeny of the stressed seeds was 1% higher. The range in yield was 3972 kg ha^{-1} to 2228 kg ha^{-1}. Standard germination of the progeny seeds grown from second generation seeds of the stress treatment increased 2% (Table 3).

Progeny seeds grown in 2011 exhibited differences in variance and in means. In plants grown from the progeny of the stressed seeds in 'BSR 101', plant stand increased by 20 plants, and the difference between the highest and lowest entries was 152 plants. Plants grown from the progeny of the stressed seed matured 3 days later than those grown from the non-stressed seeds, and the range in days to maturity was 16 days. Mean plant heights were

not different, but variance decreased in the progeny grown from the stressed seeds by 85%. Variance in the standard germination test decreased by 79% (Table 3).

In plants grown from the progeny of the stressed seeds of 'Jack', plant stand decreased by 27 plants, and variance in plant stand decreased by 67%. The range in plant stand was 280 to 158 plants. Plant heights in 'Jack' were not different, but variance in plant height decreased in the progeny grown from the stressed seeds by 87%. Yield increased by 7% in the progeny of the stressed seeds, and the difference between the highest and lowest yielding entries was 1759 kg ha^{-1}. Variance in seed oil content increased by 75% in the plants grown from the stressed seeds. Variance in seed size increased by 70% in the plants grown from the stressed seeds, and the difference in seed size between the largest and smallest entries was 5.38 g per 100 seeds. Standard germination increased by 1% for the progeny seeds grown from the second generation of the stressed seeds (Table 3).

Within cultivar variation

Changes in variance between treatments were not significant consistently across traits and cultivars, but several entries showed advantages across multiple agronomic traits and years, in both cultivars (Table 4). In 'BSR 101' entry 057 performed in the top 25% of the non-stressed or stressed treatments, and sometimes in both treatments, for yield, plant height, seed protein content, seed size, and seed standard germination and vigor. Similarly, 'BSR 101' entry 269 was in the top 25% in agronomic performance for yield, plant height, seed oil content, and seed standard germination and vigor. In the 2011 non-stressed and stressed seed treatments, 'BSR 101' entries 072 and 132 were high yielding in both treatments (Table 4).

Similar trends were observed in 'Jack'. Entries 006, 137, 166, 219, and 234 exhibited agronomic performance in the top 25% for multiple traits, including yield in all entries.

‘Jack’ entry 166 was in the top 25% of agronomic performance in all traits in the stress treatment, and was also in the top 25% for yield in the non-stress treatment. ‘Jack’ entry 219 was in the top 25% for all traits measured except seed standard germination. ‘Jack’ entry 234 was in the top 25% for protein and oil in the stress treatment (Table 4).

The same entries showed similar trends in the heritability tests, with higher agronomic performance in the same traits as those in the previous generation (Table 5). ‘BSR 101’ entries 057 was in the top 10% of agronomic performance values in yield and oil in both the 2010 and 2011 heritability tests. Entry 269 was in the top 10% of values for yield, seed oil content, seed size, and seed standard germination in 2010 (Table 5).

‘Jack’ entry 006 performed in the top 10% for yield, seed size, and seed standard germination in both 2010 and 2011. Entry 234 was in the top 10% for yield and plant height in both 2010 and 2011, and in both protein and oil in 2011 (Table 5).

Discussion

Using a modified version of the accelerated aging protocol did impose a seed stress, as evidenced by the decreased emergence of aged seeds. Accelerated aging was reported to be a seed stress (Hsu et al., 2003; Robert et al., 1979; Parrish and Leopold, 1978). In soybeans, it lowered early respiration in cotyledons, increased electrolyte leakage, decreased seed vigor (Parrish and Leopold, 1978), and decreased germination and emergence (Hsu et al., 2003; Robert et al., 1979; Parrish and Leopold, 1978). Although aging time was shortened in our experiments, there was stress on the seeds due to accelerated aging, which negatively impacted agronomic performance.

Agronomic performance also may have been negatively affected by dehydration stress associated with planting moist seeds (Guedira, et al., 1997). Wheat seeds, a

monocotyledoneous species, which were germinated for 4 d and subsequently dehydrated for 3 d had low survival rates, ranging from 60% to zero. Seedlings which grew had shorter coleoptiles lengths, and the root systems exhibited varying amounts of damage (Guedira, et al., 1997).

High temperatures and high relative humidity associated with accelerated aging were also cited as a main cause of seed deterioration (Copeland and Miller, 1995), and most species lose viability at a relative humidity greater than 80% (Toole, 1950). In maize seeds, the germination and vigor decreased due to storage at high temperature and high relative humidity (Joao and Lovato, 1999).

Due to aging at high relative humidity, the treatment imbibed seeds to around 30% moisture (Table 1), which is consistent with research by Tekrony (1995). Harrington (1972) determined that seeds with greater than 14% moisture in storage had increased respiration, which destroyed seed vigor. McDonald (1999) reported that seeds had lower viability at higher moisture contents, and that seeds at higher moisture contents were more sensitive to higher temperatures. The high temperature and relative humidity used to stress the seeds in our experiment may have contributed to decreased field vigor, and accounted for the reduction in emergence in ‘BSR 101’.

Response to the accelerated aging seed stress of 41°C and high relatively humidity immediately prior to planting was variable across cultivars and years, for changes in variance and mean responses. Differences in responses may be attributed to the effect of the seed-stress treatment. Seeds were partially imbibed at the time of planting, similar to a seed priming treatment. Seed priming is associated with water imbibition using a controlled hydration method which enables a seed to enter into phases one and two of water uptake, but

prevents them from entering into phase three (Taylor et al, 1998; Bradford and Bewley, 2002). Seeds which are partially imbibed at the time of planting are influenced by the climate and growing region (Subedi and Ma, 2005).

There were differences in climatic conditions between growing seasons (Table 6), which may have influenced the response of the partially imbibed seeds. In 2010 and 2011, plots received 2.8 mm of rainfall 96 hours after planting, and 8.9 mm of rainfall 48 hours after planting, respectively (Iowa Environmental Mesonet). Partially imbibed seeds have shown an advantage where water is limiting (Bench-Arnold and Sanchez, 2004), a factor that may account for the increases in variance, and in mean responses of traits in the 2010 growing season and may account for the variability between growing seasons. Similarly, partially imbibed seeds have shown an advantage when field conditions were suboptimal (Bench-Arnold and Sanchez, 2004; Bradford et al., 1990). Early season conditions were favorable in 2011, therefore the treatment may not have impacted the amount of variance expressed. In 2010 early season conditions were unfavorable, resulting in changes in variance and increases in agronomic performance due to the seed-stress treatment.

Genotype by environment interaction also may have contributed to differences between years and cultivars. Genotype by environment interactions in soybeans have been reported, with genotypes responding in different ways to different environments (Dashiell et al., 1994; Schultz and Bernard, 1967). Genotype by environment interactions also impacted soybean seed composition (Carver et al., 1986). Differences in growing environments, climatic conditions (Table 6), and cultivars, may account for the inconsistent responses between cultivars and growing seasons.

During 2010, there were increases in the amount of variance in ‘BSR 101 in the traits of seed oil content, seed size, and seed yield, and in ‘Jack’ in seed yield. Variation in these traits is consistent with research by Fasoula et al., (2007a; 2007b; 2007c), in which variation in the agronomic traits of seed protein, seed oil, seed weight, plant maturity, plant height, and seed yield enabled the selection of five, seven, and six new lines from within the cultivars ‘Benning’, ‘Cook’, and ‘Haskell’, respectively.

During both growing seasons, several entries of each cultivar exhibited agronomic performance in the top 25% of the non-stress and stress plots, and in the top 10% of the heritability plots (Tables 4 and 5). These entries indicate that, although not significant, variation exists within inbred soybean lines, as reported by Fasoula et al., (2007a; 2007b; 2007c), in which significant variation was used to select for new germplasm.

In 2011, there were no changes in variation in the seed-stress generation. Seed source for the experiment came from a wide-spaced, or honeycomb, planting design, which maximized phenotypic expression by maximizing genotype by environment interactions (Fasoula and Fasoula, 1997), and has been used to exploit variation in agronomic traits in soybeans, to create new lines from within cultivars, (Fasoula and Boerma, 2007). Both the non-stressed and stressed parent seeds were planted in the wide-spaced growing environment (Fig. 1). This may have allowed variation to be expressed in the seed-source generation instead of the seed-stress generation, and account for the lack of change in variance in the 2011 experiments.

De novo genetic variation has been reported in the second generation, or progeny seed, instead of the parental genotype (Rasmusson and Phillips, 1997; Lolle et al., 2005). Responses in our experiment in the non-stress and stressed generation, as well as in the

second generation heritability tests were inconsistent. In the 2010 heritability plots, there were no changes in variance. As with the parent generation, explanations may genotype by environment interactions, climatic variability, and seed source effects. In 2011, the heritability plots exhibited changes in variance in some traits, which may be attributed to *de novo* variation.

Although increases in variation in response to a seed-stress treatment were inconsistent, increases in genetic and phenotypic variation in response to plant growth environment, or plant stress have been reported (Schneeberger and Cullis, 1991; Cullis, 2005; Fasoula and Boerma, 2007; Fasoula et al., 2007). Similarly, genomic changes were not always observed in the parent genome, but in progeny plants (Rasmusson and Phillips, 1997; Lolle et al., 2005; Phillips, 2010). Further research should be conducted using a seed-stress treatment with the seed source produced in a conventional planting design, so that increases in variance can be directly attributed to the seed-stress treatment. Plant breeders may want to use these techniques to induce variation and select superior lines from established commercial cultivars to promote improved plant-stress responses in an attempt to increase yield (Johal et al., 2008; Phillip, 2010).

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Table 1. Moisture content of seeds of 'BSR 101' and 'Jack', before aging seed stress, after 24 hours of aging seed stress, and after 48 hours of aging seed stress, by using a modified accelerated aging protocol as a water imbibition method.

	Moisture Content[†]		
Cultivar	Before aging	24-hour aging	48-hour aging
'BSR 101'	0.07	0.24	0.31
'Jack'	0.07	0.26	0.33

[†] Moisture content measured by the oven dry method, at 105°C for 72 hours, and expressed as g water g^{-1} seed, on a wet weight basis.

Table 2**.** Means, variance, and maximum and minimum values for plant stand, plant height, plant maturity, seed protein and oil content, seed size, yield, and seed germination and vigor of two soybean cultivars, 'BSR 101' and 'Jack', and two growing seasons, in response to seed stress immediately before planting.

'BSR 101'

	Treatment							
Year 2010	Non-stress				Stress			
Agronomic trait	Mean	Variance	Max	Min	Mean	Variance	Max	Min
Plant height (cm)	96.72 a[z]	109.57	117.00	78.00	85.66 b	75.88	97.00	59.00
Yield (g plot^{-1})	371.02 a	360.39 B[y]	405.70	338.34	407.40 b	5625.18 A	523.96	212.95
Seed protein content (%)	35.37	0.56	36.76	34.47	35.22	1.55	37.77	33.28
Seed oil content (%)	19.25 a	0.10 B	19.80	18.58	18.65 b	0.37 A	19.41	16.94
Seed size (g 100 seeds^{-1})	14.33	0.43 B	15.41	12.96	14.34	1.29 A	16.57	12.32
Seed germination (%)	73.68 b	111.78	87.00	57.00	88.08 a	71.44	97.00	60.00
Seed vigor (%)	43.84 b	151.47	66.00	24.00	9.95 a	62.26	93.00	55.00

	Treatment							
Year 2011	Non-stress				Stress			
Agronomic trait	Mean	Variance	Max	Min	Mean	Variance	Max	Min
Plant stand (number of plants emerged)	32.34 a	19.20	44.00	20.00	26.15 b	31.06	40.00	10.00
Plant height (cm)	101.47 a	36.67	132.00	70.00	99.27 b	13.11	107.00	86.00
Maturity (days after September 1)	19.73 a	9.36	25.00	13.00	20.56 b	7.38	25.00	13.00
Yield (g plot^{-1})	579.18 a	5498.34	735.00	370.00	518.63 b	5987.68	710.00	320.00
Seed protein content (%)	34.71	1.02	37.55	32.40	34.37	0.49	37.70	32.85
Seed oil content (%)	18.74	0.11	19.50	18.00	18.91	0.13	19.90	17.90
Seed size (g 100 seeds^{-1})	16.05 a	0.47	17.59	14.48	15.89 b	0.62	17.67	10.70
Seed germination (%)	90.89	23.66	99.00	75.00	91.34	19.36	99.00	78.00
Seed vigor (%)	79.66	99.89	99.00	48.00	80.16	100.48	96.00	26.00

(continued on next page)

Table 2. Continued

'Jack'

Year 2010	Treatment							
	Non-stress				Stress			
Agronomic trait	Mean	Variance	Max	Min	Mean	Variance	Max	Min
Plant height (cm)	110.04 a	99.89 A	123.00	90.00	100.33 b	30.96 B	110.00	84.00
Yield (g plot^{-1})	385.09 b	1215.96 B	428.99	237.46	503.66 a	8426.84 A	703.93	276.93
Seed protein content (%)	35.42 b	0.44	36.98	34.36	35.77 a	0.34	37.08	34.62
Seed oil content (%)	18.57 a	0.13	19.04	17.89	18.02 b	0.06	18.50	17.50
Seed size (g 100 seeds^{-1})	11.55	0.50	13.14	10.24	11.34	0.33	12.47	10.40
Seed germination (%)	81.85 b	27.92	92.00	69.00	95.90 a	6.19	100.00	88.00
Seed vigor (%)	47.20 b	403.22	82.00	15.00	64.37 a	303.79	89.00	16.00

Year 2011	Treatment							
	Non-stress				Stress			
Agronomic trait	Mean	Variance	Max	Min	Mean	Variance	Max	Min
Plant stand (number of plants emerged)	38.60 a	18.97	49.00	26.00	37.67 b	15.83	50.00	28.00
Plant height (cm)	128.56 a	27.40	146.00	116.00	125.44 b	31.55	140.00	113.00
Maturity (days after September 1)	34.11	0.26	37.00	34.00	34.08	0.14	36.00	34.00
Yield (g plot^{-1})	728.19 a	18816.80	975.00	355.00	672.42 b	19443.69	960.00	280.00
Seed protein content (%)	34.25	0.40	36.10	33.05	34.51	0.72	37.25	32.75
Seed oil content (%)	17.89	0.12	19.25	17.10	18.00	0.14	18.70	17.00
Seed size (g 100 seeds^{-1})	13.22	0.80	16.62	11.67	13.10	0.61	15.59	11.40
Seed germination (%)	92.80	11.39	100.00	81.00	93.40	12.27	100.00	83.00
Seed vigor (%)	65.63	126.05	90.00	37.00	65.65	130.56	92.00	32.00

[z]Means within a row followed by difference lowercase letters are significantly different at $P \leq 0.05$.

[y]Variances within a row followed by different capital letters are significantly different according to Hartley's F-max test with (2,2) degrees of freedom and a critical F-value of (0.25).

Table 3. Means and variance of the means for plant stand, plant maturity, plant height, seed yield, seed protein and oil content, seed size, and seed germination and vigor, of the progeny of stressed and non-stressed seeds of two cultivars, 'BSR 101' and 'Jack', and two growing seasons.

'BSR 101'

Year 2010	Treatment							
	Non-stress				Stress			
Agronomic trait	Mean	Variance	Max	Min	Mean	Variance	Max	Min
Plant stand (number of plants emerged)	211.10 b[z]	636.29	280.00	139.00	246.21 a	555.40	280.00	190.00
Plant height (cm)	89.15	184.36	117.00	60.00	89.66	247.97	126.00	63.00
Maturity (days after September 1)	17.31 a	12.68	24.00	12.00	15.88 b	32.82	24.00	6.00
Yield (kg ha^{-1})	2941.58	69.50	3897.69	1576.95	2903.28	58.43	4087.17	1083.10
Seed protein content (%)	35.05	0.75	37.06	33.51	34.92	0.60	36.86	33.56
Seed oil content (%)	19.45	0.30	20.51	17.91	19.54	0.21	20.38	18.24
Seed size (g 100 $seeds^{-1}$)	14.05 b	0.79	16.47	11.36	14.30 a	0.72	16.44	12.01
Seed germination (%)	75.20	207.21	95.00	40.00	77.02	162.19	96.00	42.00
Seed vigor (%)	39.94	259.88	86.00	13.00	37.16	211.41	66.00	7.00
Year 2011	Treatment							
	Non-stress				Stress			
Agronomic trait	Mean	Variance	Max	Min	Mean	Variance	Max	Min
Plant stand (number of plants emerged)	182.68 b	765.96	280.00	101.00	202.08 a	698.98	280.00	128.00
Plant height (cm)	103.18	797.83 A[y]	130.67	69.33	111.36	117.16 B	133.33	82.33
Maturity (days after September 1)	22.46 b	12.74	39.00	12.00	25.61 a	26.82	33.00	17.00
Yield (kg ha^{-1})	2703.73	32.43	3786.16	104.82	2735.98	47.95	3887.61	1664.97
Seed protein content (%)	34.45	0.48	36.20	33.05	34.01	0.82	35.70	32.25
Seed oil content (%)	18.75	0.09	19.45	18.10	18.93	0.25	20.05	17.95
Seed size (g 100 $seeds^{-1}$)	16.10	0.92	17.56	12.78	16.30	1.40	18.49	14.23
Seed germination (%)	85.53	125.57 A	98.00	64.00	85.39	25.87 B	95.00	73.00
Seed vigor (%)	50.19	286.90	79.00	5.00	53.79	99.52	75.00	37.00

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Table 3. Continued

‘Jack’								
	Treatment							
Year 2010	Non-stress				Stress			
Agronomic trait	Mean	Variance	Max	Min	Mean	Variance	Max	Min
Plant stand (number of plants emerged)	213.66 b	562.60	280.00	130.00	240.09 a	654.11	280.00	133.00
Plant height (cm)	101.94	155.33	127.33	67.73	102.47	164.48	138.00	72.00
Maturity (days after September 1)	24.84 b	6.04	31.00	22.00	25.20 a	6.76	31.00	22.00
Yield (kg ha^{-1})	3329.93 b	22.10	4099.93	2066.76	3376.29 a	29.65	3972.94	2228.02
Seed protein content (%)	35.18	0.42	37.74	33.57	35.17	0.31	36.59	33.83
Seed oil content (%)	18.79	0.22	20.21	17.46	18.76	0.20	19.84	17.30
Seed size (g 100 $seeds^{-1}$)	11.42	0.74	15.68	9.37	11.42	0.47	12.72	9.19
Seed germination (%)	86.11 b	60.00	99.00	49.00	88.23 a	45.34	99.00	69.00
Seed vigor (%)	46.46	444.17	82.00	7.00	47.37	374.81	83.00	4.0
	Treatment							
Year 2011	Non-stress				Stress			
Agronomic trait	Mean	Variance	Max	Min	Mean	Variance	Max	Min
Plant stand (number of plants emerged)	254.67 a	2219.47 A	280.00	158.00	227.05 b	735.67 B	280.00	163.00
Plant height (cm)	128.46	293.42 A	152.67	88.67	132.67	38.15 B	146.67	119.33
Maturity (days after September 1)	35.25	2.33	40.00	32.00	35.28	3.34	38.00	29.00
Yield (kg ha^{-1})	3029.32 b	24.22	4234.30	2131.27	3294.33 a	19.87	4019.31	2260.94
Seed protein content (%)	34.96	0.61	36.60	33.65	34.65	0.39	36.10	33.65
Seed oil content (%)	17.75	0.07 B	18.35	17.35	17.88	0.28 A	18.70	16.70
Seed size (g 100 $seeds^{-1}$)	12.27	0.31 B	14.74	12.23	13.24	1.03 A	17.09	11.71
Seed germination (%)	92.10 b	18.73	99.00	83.00	92.71 a	17.56	99.00	82.00
Seed vigor (%)	50.02	126.08	78.00	32.00	53.02	102.89	70.00	27.70

[z]Means within a row followed by difference lowercase letters are significantly different at $P \leq 0.05$.

[y]Variances within a row followed by different capital letters are significantly different according to Hartley’s F-max test with (2,2) degrees of freedom and a critical F-value of (0.25).

Table 4. Entries with four or more agronomic performance traits in the top 25% of the cultivars 'BSR 101' and 'Jack, in 2010 and 2011, in both the non-stress and stress treatments, for yield, plant height, plant stand, plant maturity, seed protein and oil content, seed size, and seed germination and vigor.

'BSR 101'									
Year 2010			Agronomic trait						
Entry	Test	Rep	Yield (g plot^{-1})	Plant height (cm)	Seed protein content (%)	Seed oil content (%)	Seed size (g 100 seeds^{-1})	Seed germination (%)	Seed vigor (%)
BSR 101 057	Non-stress	1	**379.48**[z]	89[y]	**35.32**	19.19	**14.74**	**82**	43
	Stress	1	**523.09**	**98**	34.64	18.90	14.05	**94**	**91**
	Stress	2	442.77	87	34.56	18.72	13.89	80	**93**
BSR 101 213	Non-stress	1	359.33	**106**	**35.67**	19.18	**14.67**	57	24
	Stress	1	402.89	87	**36.45**	18.35	**16.45**	**94**	**90**
	Stress	2	212.95	59	**37.29**	18.14	**16.48**	82	80
BSR 101 269	Non-stress	1	**389.20**	**106**	34.68	**19.49**	**14.60**	**87**	**52**
	Stress	1	**483.90**	**93**	33.28	**19.24**	14.45	89	73
	Stress	2	382.05	79	34.53	18.93	13.53	84	76
BSR 101 306	Non-stress	1	**379.01**	**115**	34.52	**19.71**	**14.51**	73	**45**
	Stress	1	387.35	89	34.29	**19.20**	13.68	**95**	**83**
	Stress	2	418.56	89	34.66	**18.98**	14.28	**93**	**83**
Average[x]	Non-stress		371.02	96.72	35.37	19.25	14.33	73.68	43.84
	Stress		407.40	85.66	35.22	18.65	14.34	88.08	9.95

Year 2011			Agronomic trait								
Entry	Test	Rep	Yield (g plot^{-1})	Plant height (cm)	Plant stand (emergence)	Maturity (days after Sept. 1)	Seed protein content (%)	Seed oil content (%)	Seed size (g 100 seeds^{-1})	Seed germination (%)	Seed vigor (%)
BSR 101 072	Non-stress	1	645	**107**	33	**23**	34.10	19.10	**17.15**	96	**99**
	Non-stress	2	610	105	27	22	33.80	18.80	15.48	89	90
	Stress	1	**610**	**104**	27	22	34.50	18.80	15.55	94	88
	Stress	2	**610**	95	31	22	34.30	**19.20**	15.92	95	89
BSR 101 102	Non-stress	1	**705**	106	**39**	21	34.20	18.80	16.85	**97**	78
	Non-stress	2	540	101	**35**	**27**	34.60	18.90	16.10	91	74
	Stress	1	**655**	**103**	**39**	22	34.60	18.90	**16.80**	94	84
	Stress	2	545	101	**34**	21	34.60	19.10	16.44	95	74

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Table 4. Continued

Entry	Test	Rep	Yield (g plot^{-1})	Plant height (cm)	Plant stand (emergence)	Maturity (days after Sept. 1)	Seed protein content (%)	Seed oil content (%)	Seed size (g 100 seeds^{-1})	Seed germination (%)	Seed vigor (%)
BSR 101 132	Non-stress	1	675	102	**38**	21	34.10	18.90	16.31	**98**	88
	Non-stress	2	**720**	101	34	20	33.50	19.10	**17.20**	93	86
	Stress	1	**640**	97	26	21	34.90	18.90	**16.84**	**97**	79
	Stress	2	**630**	**104**	30	20	34.30	18.90	15.87	86	66
Average	Non-stress		579.18	101.47	32.34	19.73	34.45	18.75	16.05	90.89	79.66
	Stress		518.63	99.27	26.15	20.56	34.10	18.93	15.89	91.34	80.16

'Jack'

Year 2010			Agronomic trait							
Entry	Test	Rep	Yield (g plot^{-1})	Plant height (cm)	Seed protein content (%)	Seed oil content (%)	Seed size (g 100 seeds^{-1})	Seed germination (%)	Seed vigor (%)	
Jack 006	Non-stress	1	383.98	**116**	**36.32**	18.51	**11.99**	**83**	**66**	
	Stress	1	**569.86**	102	35.35	**18.20**	**12.43**	**99**	**85**	
	Stress	2	472.51	99	35.78	**18.13**	11.26	**98**	66	
Jack 066	Non-stress	1	**428.99**	**120**	**36.06**	18.42	**12.00**	79	**61**	
	Stress	1	**543.46**	**109**	35.57	**18.15**	**11.73**	**97**	60	
	Stress	2	467.72	96	35.60	17.90	11.36	93	60	
Jack 137	Non-stress	1	**400.27**	**122**	35.16	**18.84**	**12.63**	**83**	**65**	
	Stress	1	**594.94**	99	**36.04**	17.89	**12.64**	96	68	
	Stress	2	478.69	98	35.73	17.79	**11.85**	94	66	
Jack 166	Non-stress	1	**393.95**	71	34.99	18.60	**11.98**	81	66	
	Stress	1	**703.93**	**106**	**35.92**	**18.17**	**11.97**	**98**	**84**	
	Stress	2	476.88	97	35.69	17.90	10.97	94	66	
Jack 219	Non-stress	1	**406.27**	**116**	**35.32**	**18.85**	**13.14**	78	**77**	
	Stress	1	483.49	98	34.70	**18.29**	**11.75**	93	59	
	Stress	2	533.00	96	35.87	**18.37**	**11.90**	96	58	
Jack 234	Non-stress	1	**400.00**	**123**	35.18	**19.04**	**12.50**	79	76	
	Stress	1	**538.59**	99	**36.02**	**18.12**	**11.80**	93	**88**	
	Stress	2	458.59	99	35.67	**18.15**	11.62	93	**83**	
Average	Non-stress		385.09	110.03	35.42	18.56	11.55	81.85	47.20	
	Stress		503.66	100.33	35.77	18.01	11.34	95.90	64.37	

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Table 4. Continued

Year 2011				Agronomic trait						
Entry	Test	Rep	Yield (g plot^{-1})	Plant height (cm)	Plant stand (emergence)	Seed protein content (%)	Seed oil content (%)	Seed size (g 100 seeds^{-1})	Seed germination (%)	Seed vigor (%)
Jack 049	Non-stress	1	**910**	**136**	**45**	**35.50**	17.80	14.00	**97**	**86**
	Non-stress	2	790	118	42	34.90	17.50	13.51	93	**80**
	Stress	1	335	129	37	33.50	17.70	14.28	96	**80**
	Stress	2	**760**	121	41	34.90	**18.50**	**13.26**	94	75
Jack 131	Non-stress	1	355	**137**	34	34.00	17.20	12.62	**97**	53
	Non-stress	2	**910**	132	**46**	35.20	17.90	11.67	95	73
	Stress	1	**910**	129	37	34.10	17.40	**14.62**	96	70
	Stress	2	735	**141**	38	34.80	17.60	12.24	92	58
Average	Non-stress		728.19	129.56	38.60	34.96	17.75	13.22	92.80	65.63
	Stress		672.42	125.44	37.67	34.65	17.88	13.09	93.41	65.66

[z] Values in bold face are in the top 25% of specified trait, within the same treatment.

[y] Values which are not bold faced were not in the top 25% of the trait presented, but are provided for information.

[x] Averages presented are the averages of the treatment, not those of values presented in the table.

Table 5. Entries with four or more agronomic performance traits in the top 10% of cultivars 'Jack' and 'BSR 101', in 2010 and 2011, in a heritability test from which progeny seed was obtained in 2009 and 2010, for yield, plant height, plant stand, plant maturity, seed protein and oil content, seed size, and seed germination and vigor.

'BSR 101'										
Year 2010			Agronomic trait							
Entry	Location	Test	Yield (kg ha^{-1})	Plant height (cm)	Plant stand (plants emerged)	Seed protein content (%)	Seed oil content (%)	Seed size (g 100 seeds^{-1})	Seed germination (%)	Seed vigor (%)
BSR 101 057	Bruner	Non-stress	2645[y]	89	206	35.32	19.19	14.74	82	43
		Stress	3020	106	239	34.86	19.61	14.44	75	23
	MO Valley	Non-stress	**3476**[z]	83	219	34.01	**20.21**	14.53	**95**	17
		Stress	3043	76	**280**	33.56	**20.38**	15.15	89	56
BSR 101 081	Bruner	Non-stress	**3172**	78	220	34.84	19.59	**15.04**	74	31
		Stress	2973	99	208	34.83	**19.70**	14.72	86	34
	MO Valley	Non-stress	**3449**	85	216	34.80	19.91	14.46	80	44
		Stress	2808	83	**275**	34.51	19.91	15.38	88	34
BSR 101 269	Bruner	Non-stress	**3107**	106	211	34.68	19.41	14.60	**87**	52
		Stress	2973	107	232	34.82	**19.72**	13.89	67	54
	MO Valley	Non-stress	3241	86	234	33.71	**20.51**	**15.57**	**93**	43
		Stress	3226	73	233	33.81	**20.33**	**15.60**	86	38
BSR 101 316	Bruner	Non-stress	2828	**111**	236	35.43	19.14	14.13	**94**	**56**
		Stress	**3111**	**115**	**235**	36.05	18.50	13.43	59	27
	MO Valley	Non-stress	**3589**	80	240	34.08	**20.33**	14.21	82	14
		Stress	**3577**	86	249	33.89	20.14	13.20	91	19
Average[x]	Non-stress		2941.58	89	211.10	35.05	19.45	14.05	75.20	39.94
	Stress		2903.28	90	246.21	34.92	19.54	14.30	77.02	37.16

Year 2011			Agronomic trait								
Entry	Location	Test	Yield (kg ha^{-1})	Plant height (cm)	Plant stand (plants emerged)	Maturity (days after Sept. 1)	Seed protein content (%)	Seed oil content (%)	Seed size (g 100 seeds^{-1})	Seed germination (%)	Seed vigor (%)
BSR 101 057	Bruner	Non-stress	2312	106	**215**	29	**35.10**	18.50	**17.16**	**97**	**65**
		Stress	2542	**115**	215	**32**	33.80	19.00	15.85	79	60
	MO Valley	Non-stress	2487	99	176	**16**	33.30	**19.50**	15.59	81	42
		Stress	**3289**	**127**	**249**	**29**	33.00	**19.50**	17.34	86	57

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Table 5. Continued

Entry	Location	Test	Yield (kg ha^{-1})	Plant height (cm)	Plant stand (plants emerged)	Maturity (days after Sept. 1)	Seed protein content (%)	Seed oil content (%)	Seed size (g 100 seeds^{-1})	Seed germination (%)	Seed vigor (%)
BSR 101 081	Bruner	Non-stress	2645	107	198	26	34.70	18.40	16.21	92	**77**
		Stress	2680	111	191	**33**	32.30	**20.00**	16.29	87	**68**
	MO Valley	Non-stress	**3220**	107	**231**	24	ND	ND	15.13	77	38
		Stress	**3180**	**121**	219	18	34.50	18.80	17.31	**90**	**65**
BSR101 213	Bruner	Stress	**3480**	111	**216**	21	**35.10**	18.30	15.34	**90**	**63**
	MO Valley	Stress	2144	**121**	190	19	34.40	18.60	**17.36**	**93**	45
Average	Non-stress		2703.73	103.18	182.68	22.46	34.45	18.75	16.10	85.53	50.19
	Stress		2735.98	111.36	202.08	25.61	34.01	18.93	16.20	85.39	53.79

'Jack'

Year 2010			Agronomic trait								
Entry	Location	Test	Yield (kg ha^{-1})	Plant height (cm)	Plant stand (plants emerged)	Seed protein content (%)	Seed oil content (%)	Seed size (g 100 seeds^{-1})	Seed germination (%)	Seed vigor (%)
Jack 006	Bruner	Non-stress	2929[z]	116	208	**36.32**	18.51	11.99	83	66
		Stress	**3390**[y]	118	209	35.90	18.49	**12.70**	**89**	39
	MO Valley	Non-stress	**3665**	94	249	34.58	19.38	11.77	94	30
		Stress	3372	97	225	34.39	19.38	11.83	92	53
Jack 137	Bruner	Non-stress	3125	**122**	176	35.16	18.84	**12.64**	83	65
		Stress	3205	**124**	**257**	34.73	**19.00**	12.17	78	41
	MO Valley	Non-stress	**3613**	113	216	35.11	19.34	**12.16**	89	**71**
		Stress	**3618**	98	234	34.92	19.18	11.73	89	**76**
Jack 219	Bruner	Non-stress	3212	116	196	35.32	18.85	**13.13**	78	**77**
		Stress	2771	119	141	35.06	18.94	12.41	80	**79**
	MO Valley	Non-stress	3177	93	206	34.85	**19.66**	**12.08**	96	32
		Stress	3376	98	223	34.53	**19.74**	**12.17**	93	12
Jack 234	Bruner	Non-stress	3299	**123**	198	35.18	**19.04**	12.50	79	24
		Stress	**3401**	115	211	34.05	**19.11**	12.09	88	57
	MO Valley	Non-stress	3127	99	210	35.01	19.48	11.71	95	57
		Stress	3102	88	245	34.90	19.34	11.78	94	48
Average	Non-stress		3110.25	101.93	213.66	35.18	19.48	11.42	86.11	46.46
	Stress		3154.19	102.47	240.09	35.17	19.34	11.42	88.22	47.36

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Table 5. Continued

Year 2011			Agronomic trait								
Entry	Location	Test	Yield (kg ha^{-1})	Plant height (cm)	Plant stand (plants emerged)	Maturity (days after Sept. 1)	Seed protein content (%)	Seed oil content (%)	Seed size (g 100 seeds^{-1})	Seed germination (%)	Seed vigor (%)
Jack 006	Bruner	Non-stress	**3329**	136	200	35	33.70	17.60	**13.62**	93	44
		Stress	2864	130	219	**29**	34.40	17.70	13.30	**99**	**68**
	MO Valley	Non-stress	**3252**	131	207	**40**	34.90	17.50	**14.23**	89	46
		Stress	**3570**	**137**	240	38	34.10	18.10	**13.83**	88	50
Jack 066	Bruner	Non-stress	3173	129	253	36	34.70	17.90	12.96	95	53
		Stress	3219	135	125	35	34.60	17.30	12.15	**96**	60
	MO Valley	Non-stress	**2875**	**121**	**266**	38	35.20	**18.00**	13.09	92	50
		Stress	**3382**	**137**	**280**	34	33.70	**18.50**	**14.85**	94	31
Jack 234	Bruner	Stress	2685	143	215	35	34.20	17.90	13.39	95	**65**
	MO Valley	Stress	**3425**	**141**	252	34	**35.30**	**18.30**	**14.25**	87	40
Average	Non-stress		3029.32	128.46	254.07	35.25	34.96	17.75	12.27	92.10	50.02
	Stress		3294.33	132.67	227.05	35.28	34.65	17.35	13.24	92.71	53.02

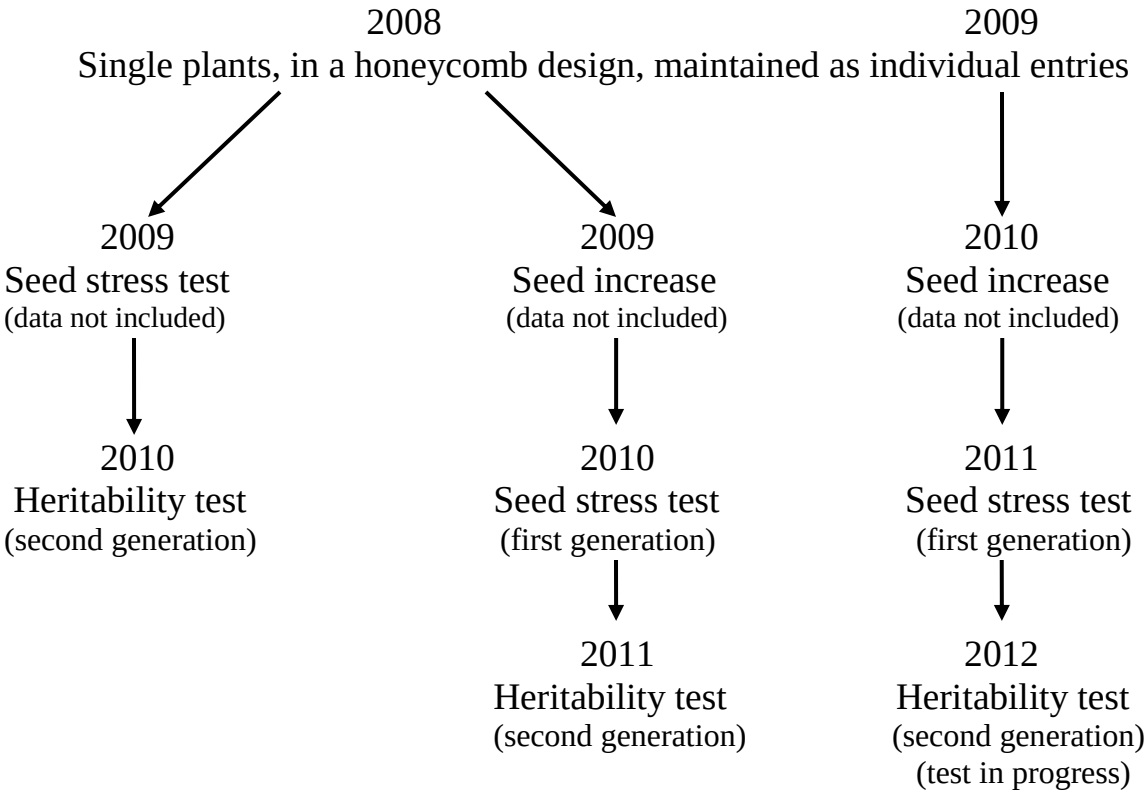
[z] Values in bold face are in the top 10% of values of the specified trait, with the same treatment, and at the same location.

[y] Values which are not bold faced were not in the top 10% of the trait presented, but are provided for information.

[x] Averages presented are the averages of the treatment, not those of values presented in the table.

Table 6. Soil types, rainfall, and temperatures, across two growing seasons, 2010 and 2011, and in two locations, near Ames, Iowa and near Missouri Valley, Iowa.

Bruner Farm, near Ames, Iowa Soil types: Clarion, Nicollet, Webster							
	Month						
Year 2010	April	May	June	July	August	September	Total
Total rainfall (mm)	99.6	88.9	311.7	121.6	396.2	124.5	1142.5
Mean temperature (°C)	13	15	22	24	24	18	
Year 2011	April	May	June	July	August	September	Total
Rainfall (mm)	100.6	142.5	160.3	74.9	75.9	43.4	597.6
Temperature (°C)	10	17	23	27	24	16	
Missouri River Valley Farm, near Missouri Valley, Iowa Soil types: Monona, Ida, Napier							
	Month						
Year 2010	April	May	June	July	August	September	Total
Total rainfall (mm)	117.6	85.1	187.7	114.8	82.3	91.1	678.6
Mean temperature (°C)	13	15	22	24	24	18	
Year 2011	April	May	June	July	August	September	Total
Total rainfall (mm)	95.8	113.5	130.8	28.7	165.8	16.3	550.9
Mean temperature (°C)	10	17	23	27	24	16	



2010 and 2011 seed stress and heritability agronomic traits measured

Plant traits
Plant height
Plant stand
Plant maturity
Seed yield

Progeny seed traits
Seed protein and oil content
Seed size
Seed germination
Seed vigor

Fig. 1. Seed source progression for the different seed-stress and heritability experiments. All seed sources originated from a single plant, in a wide-spaced, or honeycomb, planting design. The following season, seeds were either used in the stress test, or in the seed-increase plot. Following each seed-stress plot, seeds harvested from the plot were used in the heritability study.

CHAPTER 3: GENERAL CONCLUSIONS

Using a modified version of the accelerated aging protocol prior to planting decreased agronomic performance in cultivars ‘BSR 101’ and ‘Jack’ in our study. These results are evidence that the accelerated aging treatment is a form of seed stress, which is consistent with other research, and that this method could be used to induce seed stress prior to planting.

Data also showed variation present in inbred soybean lines in cultivars ‘BSR 101’ and ‘Jack’. Although increases in variance were inconsistent between years and cultivars, the range of entry-means for the agronomic traits measured was large. The range in ‘BSR 101’ seed yield for plants grown from the stressed seeds was greater than 300 g. The range in ‘Jack’ seed yield was greater than 600 g for plants grown from non-stressed and stressed seeds in one growing season, and greater than 400 g in the other growing season. ‘BSR 101’ plants grown from non-stressed and stressed seeds also showed a large range in maturity values, with a 12-day difference in days to maturity in the stressed-seed plots, and an 18 day difference in days to maturity in the second generation plots.

Additionally, some single entries from within both inbred lines consistently performed in the top 25% or top 10% in agronomic performance trait values, exhibiting positive, heritable changes in agronomic performance. For example, ‘BSR 101’ entry 057 was consistently higher yielding across multiple growing seasons, and was high in other agronomic performance traits. ‘Jack’ entry 066 was similarly consistently high yielding across multiple growing seasons and multiple locations. Additional single-plant entries from each cultivar had similar trends. This shows that although the changes in variance across treatments were not always consistent, single entries did have consistent changes in variance.

All seed in this study were originally planted in a honeycomb planting design. In our study, some of the genetic variation observed may have been the result of the honeycomb design and not the seed-stress treatment. Similarly, planting moist seeds in the field resulted in variable results due to increased sensitivity to climatic conditions, and additional dehydration stress when seeds were planted in dry soil. This may have impacted the expression of agronomic traits, and therefore, influenced the changes in variance.

Intra-cultivar variation is important to help increase plant adaptations and increase yield and other agronomic performance traits. However, further research should be conducted to separate the variation generated by seed production in a honeycomb planting design from the aging seed stress. It would be important to determine if both can be used as a way to generate intra-cultivar variation, and if one or the other has additional benefits associated with the treatment. For example, using a seed stress to generate variation may have additional value in producing plants with higher stress tolerance, due to the exposure of the seeds to stress.

The variation in agronomic performance traits of single-plant entries observed in this study generated within inbred soybean cultivars was observed in multiple growing seasons and across multiple locations. This shows that the variation is stable, and can be used to select superior plants with improved agronomic performance traits.

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APPENDIX

Additional Tables

Table 1. Means of agronomic traits of plant stand, plant height, plant maturity, yield, seed size, and seed standard germination and vigor, in response to seed stress using a modified version of the Association of Official Seed Analysis accelerated aging protocol, of 41°C for 48 hours with two varieties 'BSR 101' and 'Jack' in a side-by-side comparison, analyzed together.

	Agronomic trait								
Treatment	Emergence (no. plants)[†]	Plant height (cm)	Maturity (days after September 1)	Yield (g plot^{-1})	Seed protein content (%)	Seed oil content (%)	Seed size (g 100 seeds^{-1})	Seed germination (%)	Seed vigor (%)
Non-stressed	35.47	115.02	26.92	653.69	34.42	18.38	14.64	91.84	72.64
Stressed	31.91	112.36	27.32	595.52	34.50	18.47	14.49	92.38	72.91

[†] There was interaction between cultivar and treatment in plant emergence. When cultivars were analyzed separately, 'BSR 101 plant emergence decreased in the stressed-seeds by, on average, six plants at $P \leq 0.05$.

Table 2. Means of agronomic traits of plant stand, plant height, maturity, yield, seed size, and seed standard germination and vigor, in two cultivars 'BSR 101' and 'Jack', planted in a side-by-side comparison, analyzed separately, in response to seed stress using a modified version of the Association of Official Seed Analysis accelerated aging protocol, at 41°C for 48 hours.

	Agronomic trait						
Treatment	Plant stand (no. plants emerged)	Plant height (cm)	Maturity (days after September 1)	Yield (g plot^{-1})	Seed size (g 100 seeds^{-1})	Seed germination (%)	Seed vigor (%)
'BSR 101'							
Non-stressed	32.34 a[†]	101.47 a	19.73	579.18 a	16.05	90.89	79.66
Stressed	26.15 b	99.27 b	20.56	518.63 b	15.89	91.34	80.16
'Jack'							
Non-stressed	38.60	128.56 a	34.11	728.20 a	13.22	92.80	65.63
Stressed	37.67	125.44 b	34.08	672.42 b	13.10	93.41	65.66

[†] Values with in a row followed by different lowercase letters are significantly different at $P \leq 0.01$.

Table 3. Means and variance of the mean for plant stand, plant height, seed protein and oil content, seed size, yield and seed germination and vigor of two soybean cultivars, 'BSR 101' and 'Jack', and two growing seasons, in response to seed stress immediately before planting.

		Agronomic trait								
		Plant				Seed				
		Stand †	Maturity ‡	Height (cm)	Yield (g plot^{-1})	Size (g 100 seeds-1)	Protein Content (%)	Oil Content (%)	Germination (%)	Vigor (%)
Y 2010		CV. 'BSR 101'								
Stress	Mean	---	---	85.66 b	407.4 a	14.34	35.22	18.65 b	88.08 a	79.95 a
	Variance	---	---	75.88	5625.18 A	1.29 A	1.55	0.37 A	71.44	62.26
Non-Stress	Mean	---	---	96.72 a	371.02 b	14.33	35.37	19.25 a	73.68 b	43.84 b
	Variance	---	---	109.57	360.39 B	0.43 B	0.56	0.10 B	111.78	151.47
		CV. 'Jack'								
Stress	Mean	---	---	100.33 b	503.66 a	11.34	35.77 a	18.02 b	95.90 a	64.37 a
	Variance	---	---	30.96 B	8426.84 A	0.33	0.34	0.06	6.19 B	303.79
Non-Stress	Mean	---	---	110.04 a	385.09 b	11.55	35.42 b	18.57 a	81.85 b	47.20 b
	Variance	---	---	99.89 A	1215.96 B	0.50	0.44	0.13	27.92 A	403.22

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Table 3 Continued

Y 2011										
					CV. 'BSR 101'					
Stress	Mean	26.15 b	20.56 b	99.27 b	518.63 b	15.89 b	34.37	18.91	91.34	80.16
	Variance	31.06	7.38	13.11	5987.68	0.62	0.49	0.13	19.36	100.48
Non-Stress	Mean	32.34 a	19.73 a	101.47 a	579.18 a	16.05 a	34.71	18.74	90.89	79.66
	Variance	19.20	9.36	36.67	5498.34	0.47	1.02	0.11	23.66	99.89
					CV. 'Jack'					
Stress	Mean	37.67 b	34.08	125.44 b	627.42 b	13.10	34.51	18.00	93.40	65.65
	Variance	15.83	0.14	31.55	19443.69	0.61	0.72	0.14	12.27	130.56
Non-Stress	Mean	38.60 a	34.11	128.56 a	728.19 a	13.22	34.25	17.89	92.80	65.63
	Variance	18.97	0.26	27.40	18816.80	0.80	0.40	0.12	11.39	126.05

[z] Treatments within a column followed by difference lowercase letters are significantly different at $P \leq 0.05$.

[y]Variances within a column followed by different capital letters are significantly different according to Hartley's F-max test with (2, 2) degrees of freedom and a critical f-value of (0.25).

† Plant stand is measured as the amount of plants emerged out of 50 seeds planted.

‡ Plant maturity was measured as the number of days after September 1st when all plants in the entry were mature.

Table 4. Means and variance of the mean for plant stand, plant maturity, plant height, seed yield, seed protein and oil content, seed size, and seed germination and vigor, of the progeny of stressed and non-stressed seeds of two cultivars, 'BSR 101' and 'Jack', and two growing seasons.

		Agronomic trait								
		Plant				Seed				
		Stand †	Maturity ‡	Height (cm)	Yield (kg ha^{-1})	Size (g 100 $seeds^{-1}$)	Protein Content (%)	Oil Content (%)	Germination (%)	Vigor (%)
Y 2010					CV. 'BSR 101'					
Stress	Mean	246.21 a	15.88 b	89.66	2903.28	14.30 a	34.92	19.54	77.02	37.16
	Variance	555.40	32.82	247.97	58.43	0.72	0.60	0.21	162.19	211.41
Non-Stress	Mean	211.10 b	17.31 a	89.15	2941.58	14.05 b	35.05	19.45	75.20	39.94
	Variance	636.29	12.68	184.36	69.50	0.79	0.75	0.30	207.21	259.88
					CV. 'Jack'					
Stress	Mean	240.09 a	25.20 a	102.47	3376.29 a	11.42	35.17	18.76	88.23 a	47.37
	Variance	654.11	6.76	164.48	29.65	0.47	0.31	0.20	45.34	374.81
Non-Stress	Mean	213.66 b	24.84 b	101.94	3329.93 b	11.42	35.18	18.79	86.11 b	46.46
	Variance	562.60	6.04	155.33	22. 10	0.74	0.42	0.22	60.00	444.17

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Table 4 Continued

Y 2011						CV. 'BSR 101'				
Stress	Mean	202.82 a	25.61 a	111.36	2735.98	16.30	34.01	18.93	85.39	53.79
	Variance	698.98	26.82	117.16 B	47.95	1.40	0.82	0.25	25.87 B	99.52
Non-Stress	Mean	182.68 b	22.46 b	103.18	2703. 73	16.10	34.45	18.75	85.53	50.19
	Variance	765.96	12.74	797.83 A	32.43	0.92	0.48	0.09	125.57 A	286.90
						CV. 'Jack'				
Stress	Mean	227.05 b	35.28	132.67	3294.33	13.24	34.65	17.88	92.71 a	53.02
	Variance	735.67 B	3.34	38.15 B	19.87	1.03 A	0.39	0.28 A	17.56	102.89
Non-Stress	Mean	254.67 a	35.25	128.46	3029.32	12.27	34.96	17.75	92.10 b	50.02
	Variance	2219.47 A	2.33	293.42 A	24.22	0.31 B	0.61	0.07 B	18.73	126.08

[z]Treatments within a column followed by difference lowercase letters are significantly different at $P \leq 0.05$.

[y]Variances within a column followed by different capital letters are significantly different according to Hartley's F-max test with (2, 2) degrees of freedom and a critical f-value of (0.25).

† Plant stand is measured as the amount of plants emerged per plot

‡ Plant maturity was measured as the number of days after September 1st when all plants in the entry were mature

Table 5. Entries with four or more agronomic performance traits in the bottom 25% of the cultivars 'BSR 101' and 'Jack, in 2010 and 2011, in both the non-stress and stress treatments, for yield, plant height, plant stand, plant maturity, seed protein and oil content, seed size, and seed germination and vigor.

'BSR 101'										
Year 2010			Agronomic trait							
Entry	Test	Rep	Yield (g plot^{-1})	Plant height (cm)	Seed protein content (%)	Seed oil content (%)	Seed size (g 100 seeds^{-1})	Seed germination (%)	Seed vigor (%)	
BSR 101 068	Non-stress	1	**344**[z]	**89**	36.27	**18.97**	14.55	**58**	**38**	
	Stress	1	509[y]	83	36.36	**17.91**	16.51	90	82	
	Stress	2	**278**	**69**	36.35	18.31	14.83	**82**	90	
BSR 101 071	Non-stress	1	370	97	35.85	**19.14**	14.85	**61**	**38**	
	Stress	1	396	97	**33.94**	19.39	**12.88**	94	**75**	
	Stress	2	451	97	**34.19**	**19.09**	**13.26**	**64**	**67**	
BSR 101 159	Non-stress	1	**340**	**80**	36.76	**18.58**	**12.86**	**71**	**34**	
	Stress	1	**307**	81	37.38	**18.96**	**13.43**	87	84	
	Stress	2	**348**	**73**	**34.87**	**16.94**	13.96	92	88	
BSR 101 289	Non-stress	1	**344**	96	36.29	19.34	14.61	**59**	**43**	
	Stress	1	**308**	85	36.30	**18.18**	**13.65**	**81**	79	
	Stress	2	**307**	**74**	37.77	**17.52**	15.40	**60**	88	
Average[x]	Non-stress		371.02	96.72	35.37	19.25	14.33	73.68	43.84	
	Stress		407.40	85.66	35.22	18.65	14.34	88.08	9.95	

Year 2011			Agronomic trait								
Entry	Test	Rep	Yield (g plot^{-1})	Plant height (cm)	Plant stand (emergence)	Maturity (days after Sept. 1)	Seed protein content (%)	Seed oil content (%)	Seed size (g 100 seeds^{-1})	Seed germination (%)	Seed vigor (%)
BSR 101 059	Non-stress	1	**525**	**98**	**28**	22	34.40	19.00	16.29	92	76
	Non-stress	2	645	100	**28**	**17**	35.20	18.70	16.49	92	91
	Stress	1	**345**	**86**	**15**	21	35.00	**18.70**	15.90	93	86
	Stress	2	485	**94**	**19**	22	34.80	**18.50**	**15.31**	93	95
BSR 101 106	Non-stress	1	**520**	101	**27**	21	34.90	18.80	15.65	89	86
	Non-stress	2	530	**96**	**28**	**15**	**33.30**	19.30	15.71	89	**58**
	Stress	1	**445**	**93**	**19**	**13**	34.30	19.30	**15.13**	91	81
	Stress	2	535	**94**	33	**15**	35.00	19.10	16.89	**84**	83

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Table 5. Continued

Entry	Test	Rep	Yield (g plot^{-1})	Plant height (cm)	Plant stand (emergence)	Maturity (days after Sept. 1)	Seed protein content (%)	Seed oil content (%)	Seed size (g 100 seeds^{-1})	Seed germination (%)	Seed vigor (%)
BSR 101 117	Non-stress	1	560	101	**25**	19	**32.90**	19.30	15.90	**86**	90
	Non-stress	2	580	102	**29**	22	**33.50**	19.20	16.01	**83**	**58**
	Stress	1	**430**	**89**	**14**	21	34.40	19.00	15.71	**86**	84
	Stress	2	**320**	**92**	**14**	22	34.70	19.10	**14.95**	**81**	**62**
BSR 101 150	Non-stress	1	**455**	**99**	31	**15**	35.10	18.90	16.20	95	**63**
	Non-stress	2	**370**	**93**	**26**	**16**	34.90	19.90	16.15	**80**	77
	Stress	1	510	98	**23**	20	35.40	**18.40**	15.66	91	**73**
	Stress	2	500	**96**	**19**	**15**	35.30	**18.70**	16.22	**84**	**71**
Average	Non-stress		579.18	101.47	32.34	19.73	34.45	18.75	16.05	90.89	79.66
	Stress		518.63	99.27	26.15	20.56	34.10	18.93	15.89	91.34	80.16

'Jack'

Year 2010			Agronomic trait							
Entry	Test	Rep	Yield (g plot^{-1})	Plant height (cm)	Seed protein content (%)	Seed oil content (%)	Seed size (g 100 seeds^{-1})	Seed germination (%)	Seed vigor (%)	
Jack 152	Non-stress	1	**375**	106	35.56	**17.94**	**10.47**	**88**	**17**	
	Stress	1	**366**	**85**	36.48	**17.83**	**10.99**	**88**	73	
	Stress	2	**276**	97	36.16	**17.65**	**10.58**	96	74	
Jack 182	Non-stress	1	**396**	**102**	35.92	**17.89**	**10.95**	**82**	45	
	Stress	1	601	110	36.18	**17.51**	**10.63**	96	**34**	
	Stress	2	**371**	109	36.25	**17.64**	**10.62**	**93**	62	
Jack 184	Non-stress	1	392	**100**	36.98	**18.04**	11.25	**83**	36	
	Stress	1	588	**93**	36.90	18.06	11.63	98	56	
	Stress	2	**326**	104	36.97	**17.94**	**10.61**	**92**	84	
Jack 204	Non-stress	1	**273**	110	**34.98**	18.68	11.23	**86**	**25**	
	Stress	1	**418**	**95**	35.79	**17.69**	11.95	98	73	
	Stress	2	547	**96**	35.50	17.95	**11.23**	94	80	
Average	Non-stress		385.09	110.03	35.42	18.56	11.55	81.85	47.20	
	Stress		503.66	100.33	35.77	18.01	11.34	95.90	64.37	

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Table 5. Continued

Year 2011				Agronomic trait						
Entry	Test	Rep	Yield (g plot^{-1})	Plant height (cm)	Plant stand (emergence)	Seed protein content (%)	Seed oil content (%)	Seed size (g 100 seeds^{-1})	Seed germination (%)	Seed vigor (%)
Jack 098	Non-stress	1	**655**	**125**	44	34.40	17.90	**11.98**	96	88
	Non-stress	2	910	**123**	39	35.10	**17.70**	**12.08**	92	60
	Stress	1	600	**118**	36	35.10	18.20	**11.40**	**88**	62
	Stress	2	660	**116**	44	35.00	17.80	12.56	97	74
Jack 140	Non-stress	1	715	**125**	36	**33.80**	18.40	13.78	**88**	55
	Non-stress	2	730	126	**35**	34.80	17.80	13.62	94	54
	Stress	1	**485**	**119**	**30**	34.70	**17.10**	13.22	**89**	78
	Stress	2	755	128	**28**	**33.10**	18.10	14.31	94	**46**
Average	Non-stress		728.19	129.56	38.60	34.96	17.75	13.22	92.80	65.63
	Stress		672.42	125.44	37.67	34.65	17.88	13.09	93.41	65.66

[z] Values in bold face are in the top 25% of values of the specified trait, within the same treatment.

[y] Values which are not bold faced were not in the top 25% of the trait presented, but are provided for information.

[x] Averages presented are the averages of the treatment, not those of values presented in the table.

Table 6. Entries with four or more agronomic performance traits in the bottom 10% of cultivars 'Jack' and 'BSR 101', in 2010 and 2011, in a heritability test from which progeny seed was obtained in 2009 and 2010, for yield, plant height, plant stand, plant maturity, seed protein and oil content, seed size, and seed germination and vigor.

'BSR 101'										
Year 2010			Agronomic trait							
Entry	Location	Test	Yield (kg ha^{-1})	Plant height (cm)	Plant stand (plants emerged)	Seed protein content (%)	Seed oil content (%)	Seed size (g 100 seeds^{-1})	Seed germination (%)	Seed vigor (%)
BSR 101 068	Bruner	Non-stress	**2283**[z]	**89**	232	36.27	**18.97**	14.55	**58**	38
		Stress	2627[y]	99	241	35.91	19.39	14.95	74	**22**
	MO Valley	Non-stress	**1978**	**62**	203	35.39	19.30	**12.92**	**58**	32
		Stress	2126	**70**	241	35.24	19.75	13.69	**65**	35
BSR 101 159	Bruner	Non-stress	**2025**	**80**	218	36.70	**18.58**	**12.86**	71	34
		Stress	2610	109	243	34.75	19.35	14.14	83	60
	MO Valley	Non-stress	2095	**68**	171	35.39	**18.65**	**12.26**	80	42
		Stress	**1399**	**71**	226	34.99	**19.25**	**12.01**	77	**18**
BSR 101 289	Bruner	Non-stress	**2271**	96	241	36.29	19.34	14.61	**59**	43
		Stress	2568	94	245	36.72	**18.50**	14.53	**50**	**20**
	MO Valley	Non-stress	2816	91	230	35.37	19.70	13.29	**57**	30
		Stress	2420	**68**	226	34.79	19.99	**12.99**	72	39
Average[x]	Non-stress		2941.58	89	211.10	35.05	19.45	14.05	75.20	39.94
	Stress		2903.28	90	246.21	34.92	19.54	14.30	77.02	37.16

Year 2011			Agronomic trait								
Entry	Location	Test	Yield (kg ha^{-1})	Plant height (cm)	Plant stand (plants emerged)	Maturity (days after Sept. 1)	Seed protein content (%)	Seed oil content (%)	Seed size (g 100 seeds^{-1})	Seed germination (%)	Seed vigor (%)
BSR 101 048	Bruner	Non-stress	**2173**	99	**141**	**12**	34.70	18.50	16.99	83	56
		Stress	2607	107	217	**21**	**33.50**	19.30	17.44	**87**	**49**
	MO Valley	Non-stress	**2025**	**85**	**156**	**16**	34.30	18.90	15.97	97	79
		Stress	3021	127	201	18	34.20	18.90	16.09	**83**	**5**

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Table 6. Continued

Entry	Location	Test	Yield (kg ha^{-1})	Plant height (cm)	Plant stand (plants emerged)	Maturity (days after Sept. 1)	Seed protein content (%)	Seed oil content (%)	Seed size (g 100 seeds^{-1})	Seed germination (%)	Seed vigor (%)
BSR 101 078	Bruner	Non-stress	2592	97	213	26	ND	ND	16.87	ND	ND
		Stress	2420	**93**	**128**	23	35.30	18.00	**15.42**	**80**	**52**
	MO Valley	Non-stress	3007	104	174	**16**	34.10	19.20	**15.13**	**77**	**38**
		Stress	2692	105	160	22	34.00	18.00	**15.43**	**80**	**38**
BSR101 099	Bruner	Stress	2425	**95**	189	21	**33.10**	18.90	**15.85**	**67**	**33**
	MO Valley	Stress	2588	98	212	21	34.50	19.10	**14.19**	**85**	**42**
Average	Non-stress		2703.73	103.18	182.68	22.46	34.45	18.75	16.10	85.53	50.19
	Stress		2735.98	111.36	202.08	25.61	34.01	18.93	16.20	85.39	53.79

'Jack'

Year 2010			Agronomic trait							
Entry	Location	Test	Yield (kg ha^{-1})	Plant height (cm)	Plant stand (plants emerged)	Seed protein content (%)	Seed oil content (%)	Seed size (g 100 seeds^{-1})	Seed germination (%)	Seed vigor (%)
Jack 010	Bruner	Non-stress	**2823**	115	205	**34.90**	18.71	**10.24**	**69**	65
		Stress	3288	**98**	235	**34.96**	**18.38**	**11.14**	**80**	57
	MO Valley	Non-stress	3269	**94**	211	**34.97**	**18.41**	**9.87**	93	56
		Stress	3280	**99**	233	35.52	18.72	**10.27**	94	**14**
Jack 112	Bruner	Non-stress	3183	106	186	36.16	**18.00**	**10.89**	**75**	**15**
		Stress	3294	109	245	**34.73**	**18.35**	11.69	**87**	44
	MO Valley	Non-stress	**3165**	**94**	228	**35.20**	18.91	**10.24**	**89**	71
		Stress	**3165**	**90**	234	**34.95**	18.89	**10.66**	**89**	35
Jack 127	Bruner	Non-stress	**2067**	**101**	199	35.73	18.56	**9.98**	93	39
		Stress	3051	112	214	36.16	**18.35**	11.80	**78**	**26**
	MO Valley	Non-stress	3386	**89**	241	35.37	18.55	**10.62**	92	51
		Stress	**2967**	**85**	222	35.59	**18.38**	**10.26**	95	58
Average	Non-stress		3110.25	101.93	213.66	35.18	19.48	11.42	86.11	46.46
	Stress		3154.19	102.47	240.09	35.17	19.34	11.42	88.22	47.36

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Table 6. Continued

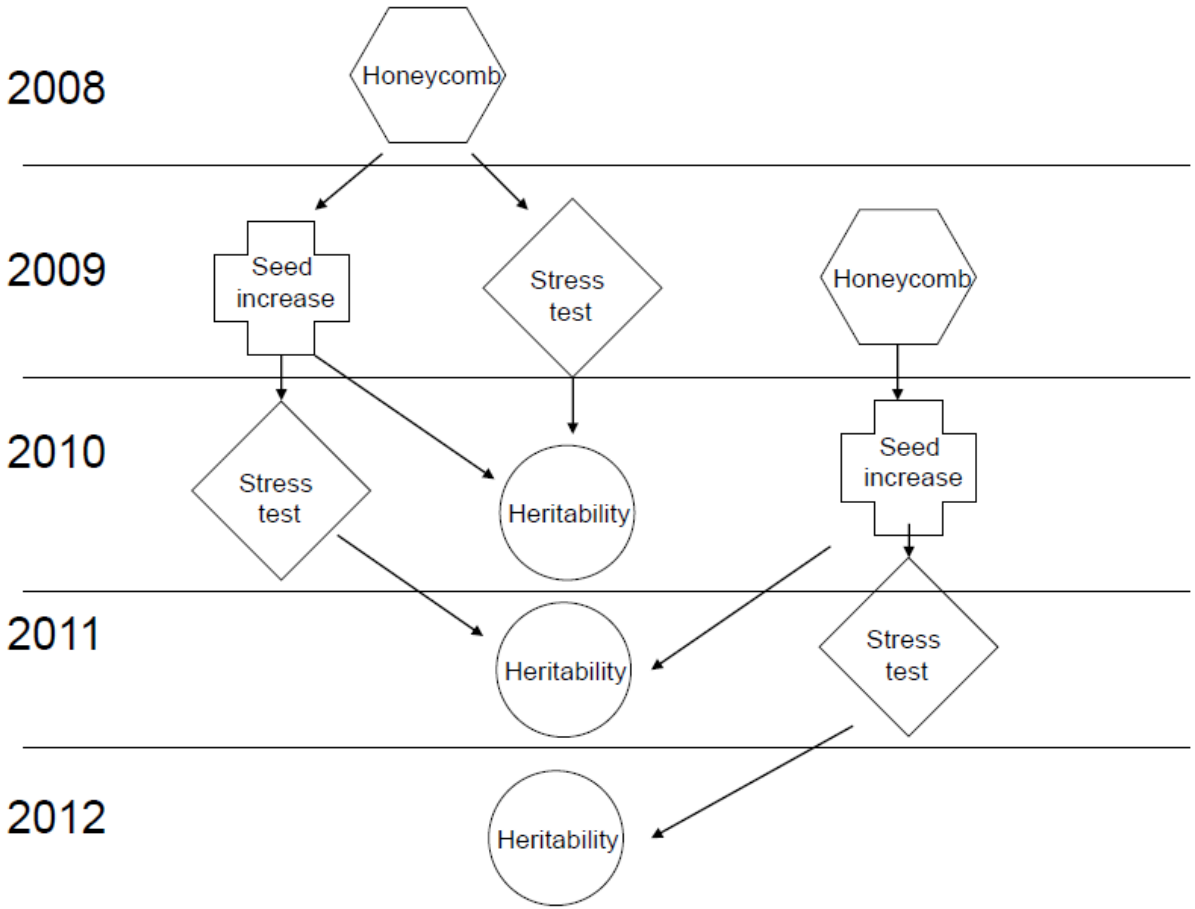
Year 2011			Agronomic trait								
Entry	Location	Test	Yield (kg ha^{-1})	Plant height (cm)	Plant stand (plants emerged)	Maturity (days after Sept. 1)	Seed protein content (%)	Seed oil content (%)	Seed size (g 100 seeds^{-1})	Seed germination (%)	Seed vigor (%)
Jack 066	Bruner	Non-stress	2987	127	200	35	34.70	17.90	12.96	95	53
		Stress	3446	135	**175**	34	34.60	**17.30**	**12.15**	96	**51**
	MO Valley	Non-stress	**2380**	121	243	35	35.20	18.00	13.90	**92**	**50**
		Stress	3374	137	200	33	**33.70**	18.50	13.15	95	57
Jack 112	Bruner	Non-stress	3080	126	223	35	**33.70**	18.30	13.40	97	68
		Stress	3249	136	**178**	35	**34.10**	**17.40**	**12.59**	95	**51**
	MO Valley	Non-stress	2882	127	**190**	34	36.30	**17.80**	13.11	92	**50**
		Stress	3172	139	232	33	**33.90**	18.60	13.24	95	**43**
Jack 137	Bruner	Non-stress	2941	127	251	38	35.00	**17.60**	13.65	92	52
		Stress	3112	135	220	35	**34.30**	**17.60**	13.43	95	**47**
	MO Valley	Non-stress	2585	125	268	37	36.50	**17.50**	13.04	**83**	**32**
		Stress	22778	129	210	36	**34.80**	17.90	13.94	93	54
Average	Non-stress		3029.32	128.46	254.07	35.25	34.96	17.75	12.27	92.10	50.02
	Stress		3294.33	132.67	227.05	35.28	34.65	17.35	13.24	92.71	53.02

[z] Values in bold face are in the top 10% of values of the specified trait, within the same treatment and at the same location.

[y] Values which are not bold faced were not in the top 10% of the trait presented, but are provided for information.

[x] Averages presented are the averages of the treatment, not those of values presented in the table.

Fig. 1. Seed source progression diagram, contributed by Dr. Susan Lolle. All seed sources originated from a single plant, in a wide-spaced, or honeycomb, planting design. The following season, seeds were either used in the stress test, or in the seed-increase plot. Following each seed-stress plot, seeds harvested from the plot were used in the heritability study.



Additional Information

Statistical Design

Non-stressed and stressed plot set-up: split-plot field design

Rep 1				Rep 2			
Jack		BSR		Jack		BSR	
ST	Non-ST	ST	Non-ST	ST	Non-ST	ST	Non-ST

Linear Additive Model

$\mathbf{Y_{ij} = \mu + R_i + \delta_{(i)} + T_j + RT_{ij}}$

Where R = rep and T = treatment

*Cultivars analyzed separately to account for all within cultivar variation

ANOVA

		2	2	
		R	F	
Source	DF	i	j	EMS
R_i	1	1	2	$\sigma^2 + 2\sigma^2\delta + 2\sigma^2 R$
$\delta_{(i)}$	0	1	2	$\sigma^2 + 2\sigma^2\delta$
T_j	1	2	0	$\sigma^2 + \sigma^2 RT + 2\varphi T$
RT_{ij}	1	1	0	$\sigma^2 + \sigma^2 RT$

Hertitability plot set-up: split-plot field design

Ames								Missouri Valley							
Block 1				Block 2				Block 1				Block 2			
Jack		BSR101		Jack		BSR101		Jack		BSR101		Jack		BSR101	
P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP
R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R

Linear Additive Models

- Traits with two reps of data collected at both locations

  $\mathbf{Y_{ijk} = \mu + L_i + B_{(i)j} + + T_k + LT_{ik} + BT_{(i)jk}}$

  L = Location, B = Block or Rep, T = Treatment

		2	2	2	
		R	R	F	
Source	DF	i	j	k	EMS
L_i	1	1	2	2	$\sigma^2 + 2\sigma^2 B + 4\sigma^2 L$
$B_{(i)j}$	2	1	1	2	$\sigma^2 + 2\sigma^2 B$
T_k	1	2	2	0	$\sigma^2 + \sigma^2 BT + 2\sigma^2 LT + 4\varphi T$
LT_{ik}	1	1	2	0	$\sigma^2 + \sigma^2 BT + 2\sigma^2 LT$
$BT_{(i)jk}$	2	1	1	0	$\sigma^2 + \sigma^2 BT$

- Traits with one rep of data collected at each location (repetition is in the location term)
  $\mathbf{Y_{ij} = \mu + L_i + \delta_{(i)} + T_j + LT_{ij}}$
  L = Location, T = Treatment

		2	2	
		R	F	
Source	DF	i	j	EMS
L_i	1	1	2	$\sigma^2 + 2\,\sigma^2\,\delta + 2\,\sigma^2 L$
$\delta_{(i)}$	0	1	2	$\sigma^2 + 2\,\sigma^2\,\delta$
T_j	1	2	0	$\sigma^2 + \sigma^2 LT + 2\varphi T$
LT_{ij}	1	1	0	$\sigma^2 + \sigma^2 LT$